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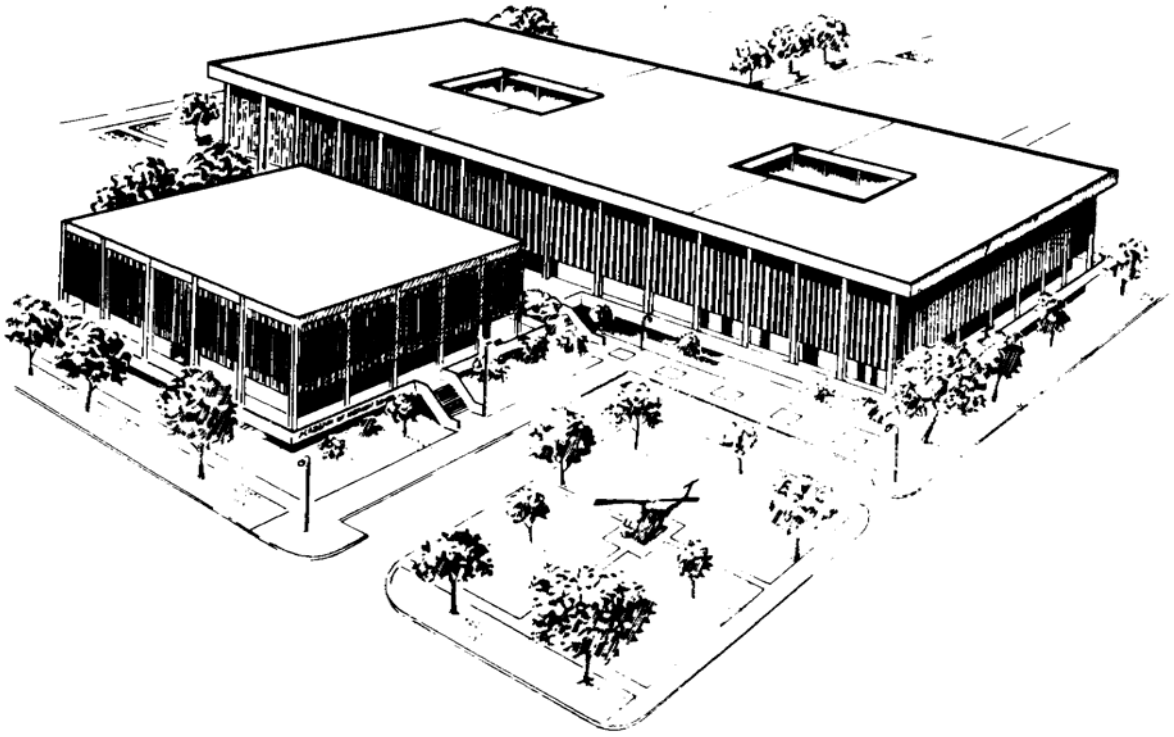
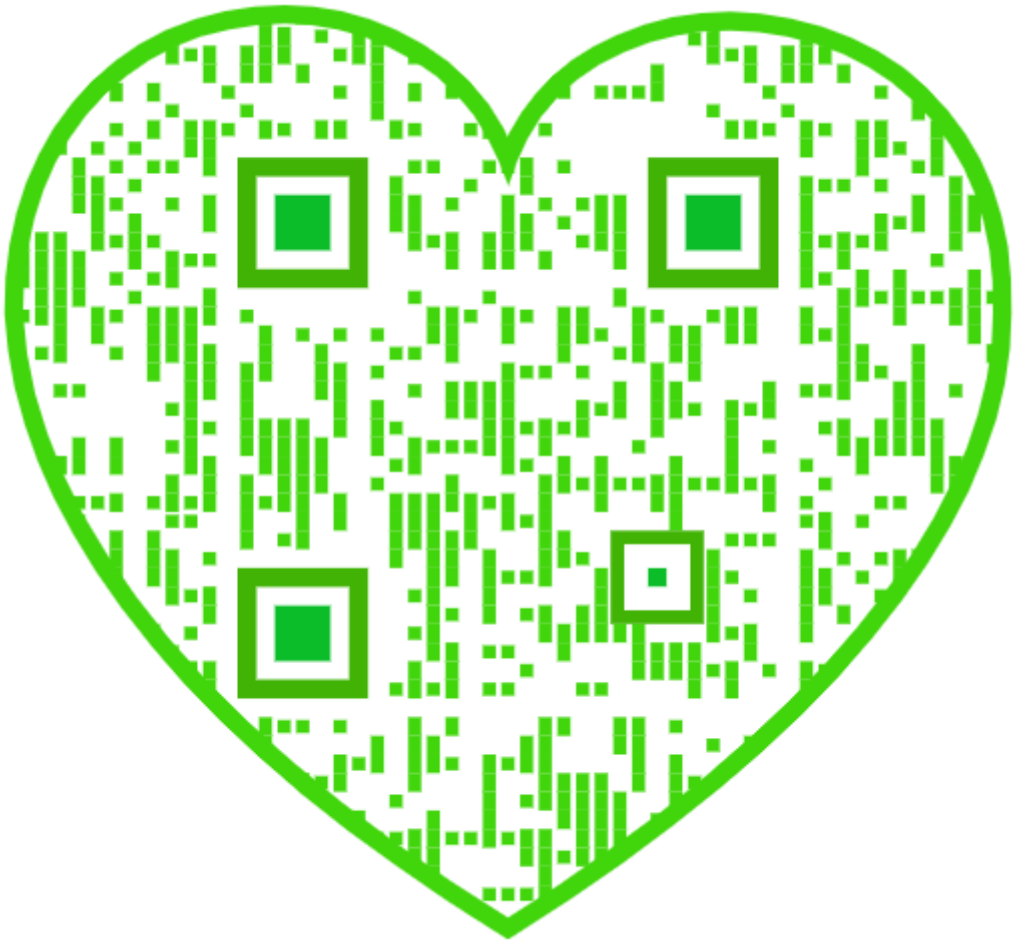


PRINCIPLES OF EPIDEMIOLOGY AND MICROBIOLOGY

SUBCOURSE MD0151 EDITION 100



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CLARIFICATION OF TRAINING LITERATURE TERMINOLOGY

When used in this publication, words such as "he," "him," "his," and "men" are intended to include both the masculine and feminine genders, unless specifically stated otherwise or when obvious in context.
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PRINCIPLES OF EPIDEMIOLOGY AND MICROBIOLOGY

INTRODUCTION

One of the important landmarks in man's struggle to conquer disease was the invention of the microscope, generally attributed to Anton van Leeuwenhoek of Holland. Leeuwenhoek described the appearance of protozoa to the Royal Society of London in 1673. Thus, the world became aware of microbial life. Subsequently, Louis Pasteur, Lord Lister, Robert Koch, and others established the role of microorganisms as the causative agents of many diseases and developed techniques for determining the etiology (cause) and preventive measures for many previously uncontrolled diseases.

During the period 1893--1902, several important events occurred in military medicine that benefited not only the Army, but mankind in general. Under the direction of Army Surgeon General George Sternberg (also known as "The Father of American Bacteriology"), Walter Reed, William Gorgas, and other medical officers made dramatic progress in the etiology and control of typhoid fever, malaria, and yellow fever.

This subcourse introduces the basic principles of disease transmission and epidemiology--principles which were used by Reed and Gorgas and which are in use today. It also introduces the student to the study of the microbiological agents, which are important from a military and public health viewpoint.

Subcourse Components:

This subcourse consists of three lessons.

Lesson 1, Introduction to Disease Transmission and Epidemiology.

Lesson 2, Public Health Microbiology.

Lesson 3, Practical Application of Microbiology

Study Suggestions:

Here are some suggestions that may be helpful to you in completing this subcourse:

--Read and study each lesson carefully.

--Complete the subcourse lesson by lesson. After completing each lesson, work the exercises at the end of the lesson, marking your answers in this booklet.

--After completing each set of lesson exercises, compare your answers with those on the solution sheet that follows the exercises. If you have answered an exercise incorrectly, check the reference cited after the answer on the solution sheet to determine why your response was not the correct one.

Credit Awarded:

To receive credit hours, you must be officially enrolled and complete an examination furnished by the Nonresident Instruction Branch at Fort Sam Houston, Texas. Upon successful completion of the examination for this subcourse, you will be awarded 5 credit hours.

You can enroll by going to the web site http://atrrs.army.mil and enrolling under "Self Development" (School Code 555).

A listing of correspondence courses and subcourses available through the Nonresident Instruction Section is found in Chapter 4 of DA Pamphlet 350-59, Army Correspondence Course Program Catalog. The DA PAM is available at the following website: http://www.usapa.army.mil/pdffiles/p350-59.pdf.

LESSON ASSIGNMENT

LESSON 1	Introduction to Disease Transmission and Epidemiology.
TEXT ASSIGNMENT	Paragraphs 1-1 through 1-13
LESSON OBJECTIVES	After completing this lesson, you should be able to:

1-1. Identify the links in the chain of disease transmission and select an appropriate means of breaking each link.

1-2. When given examples of common diseases and the accompanying factors, identify those elements that illustrate the principles of "multiple causation" and "iceberg effect."

1-3. Select from a list of hypothetical situations those elements of data that have value from an epidemiologic viewpoint.

1-4. Identify the major uses of the epidemiology in preventive medicine.

SUGGESTION After completing the assignment, complete the exercises of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 1

INTRODUCTION TO DISEASE TRANSMISSION AND EPIDEMIOLOGY

1-1. GENERAL

Disease and non-combat injury have plagued military commanders since time immemorial. In every war or combat action in which the United States (US) has ever participated, disease and non-combat injury have contributed far more to the ineffectiveness of troops than have losses due to enemy action. A commonly held misconception is that since the advent of antibiotics and advanced medical techniques, disease and accident are no longer matters of major concern. Nothing could be farther from the truth. Statistics show that during the heaviest periods of combat action since the outbreak of World War II, hospital admissions caused by disease and non-combat injury have exceeded those caused by battle injury by a ratio of from 3:1 to 19:1 (Table 1-1). Prior to World War II, these ratios were even less favorable. Much credit for the improvement in the situation must be given to epidemiologists who discovered the causes of many of the diseases that had ravaged troops for centuries. As more is learned in the future, the number of needless deaths and illnesses will hopefully be further reduced. The key to opening the door to further discovery of methods of disease control is a thorough knowledge of the various agents of disease and the nature of disease transmission.

War Period and Location	Disease and Non-Combat Injury	Battle Injury
Pacific Theater of Operations Nov 1942 to Aug 1945 (WWII)	95%	5%
European Theater of Operations June 1944 to May 1945 (WWII)	77%	23%
Korean War July 1950 to July 1953	83%	17%
Vietnam War 1 Jan 1969 to 31 Dec 1969	81.8%	18.2%

Table 1-1. US Army hospital admissions during selected war periods.

1-2. RESPONSIBILITIES FOR HEALTH

The commander of a military organization is responsible for the health of his command. In the fulfillment of this responsibility, he is assisted by a staff of trained specialists. The surgeon, who is the chief medical advisor to the commander, provides technical medical advice and is responsible for the successful functioning of the medical service within the command. Additional support is readily available from field medical treatment facilities, Army medical laboratories, Medical Department table of organization and equipment (TOE) units, and facilities of The Surgeon General's office. Emphasis throughout the Army at all levels is on prevention of disease, using all available information concerning the epidemiology of those diseases which are of military or public health significance.

1-3. DEFINITIONS

In order to approach the subject of disease transmission and epidemiology in a clear and logical manner, it is necessary to establish a common ground in the use of terminology. The following terms will be used frequently throughout this subcourse.

a. **Disease**. Disease is an impairment of the normal state of the living animal or plant body that affects the performance of the vital functions. The presence of disease usually results in visible signs or symptoms.

b. **Communicable Disease**. A communicable disease is an illness that can be transmitted person to person or from animal to person.

c. **Agent**. An agent is a disease-producing organism or substance.

d. **Infection**. Infection is the entrance and multiplication of infectious (disease-producing) agents into the body of man or animal.

e. **Reservoir**. A reservoir is the source of a disease, harboring the infectious agent(s). The agent either multiplies or undergoes some development with the organism or substance acting as the reservoir.

f. **Mode of Transmission**. The mode of transmission is the means by which a disease is transmitted from one person or animal to another.

g. **Vector**. A vector is an animal or arthropod that plays a part in the transmission of disease. A disease vector may be either the reservoir or the vehicle in disease transmission.

h. **Host**. The host is the living body upon which a parasite or infectious agent lives--the final recipient of a disease agent. The host of a disease may be either a case or a carrier.

i. **Case**. A case refers to a person who is actually ill with a disease.

j. **Carrier**. A carrier is an individual (or animal) who is infected with a disease, agent and is capable of transmitting the disease, but who usually does not exhibit clinical symptoms.

k. **Incubation Period**. The incubation period is the time interval between the entrance of an infectious agent into a host and the appearance of symptoms.

l. **Spectrum of Infection**. The spectrum of infection is the broad gradation of disease infection from no apparent symptoms (such as the carrier state) through severe illness and death.

m. **Endemic**. Endemic refers to the usual level of occurrence of a disease within a given geographical area.

n. **Epidemic**. Epidemic is the occurrence of a disease clearly in excess of the normal expectancy within a given geographical area.

o. **Pandemic**. Pandemic is the occurrence of disease over a wide geographical area and affecting an exceptionally high percentage of the population.

p. **Epidemiology**. Epidemiology is the study of the determinants and distribution of disease and injury in a given population.

q. **Vehicle**. A vehicle is an inanimate object that facilitates the transmission of a disease-causing agent.

1-4. COMMUNICABLE DISEASES

Although not all diseases of military importance are communicable, this lesson will focus upon the communicable diseases-those that can be transmitted from person to person or from an animal to a person. These diseases may be classified into five groups, based upon the manner in which they are spread, the area of the body that they affect, and the type of control needed to prevent their spread.

a. **Intestinal Diseases**. These diseases are usually transmitted by food or water that has become contaminated with feces from an infected human or animal. Examples are typhoid and paratyphoid fevers, dysentery, and cholera.

b. **Respiratory Diseases**. These diseases are usually transmitted from person to person by discharges from the nose, mouth, throat, or lungs of an infected person. Examples are the common cold, influenza, pneumonia, streptococcal sore throat, and tuberculosis.

c. **Sexually Transmitted Diseases**. These diseases are transmitted from person to person by sexual intercourse. Examples are syphilis, gonorrhea, herpes, hepatitis B, and chancroid.

d. **Arthropod-Borne Diseases**. These diseases are transmitted from person to person or from animal to person by insects or other arthropods. Examples are malaria, typhus, and yellow fever.

e. **Miscellaneous Diseases**. This group includes those communicable diseases that do not fall into any of the above groups. Examples are rabies (hydrophobia), tetanus (lockjaw), and dermatophytosis (athlete's foot).

1-5. THE CHAIN OF DISEASE TRANSMISSION

a. Each case of communicable disease is the result of an orderly progression in a series of events. This series of events may be described as a three-link chain, each link representing a factor essential to the transmission of disease. These links (figure 1-1) are:

(1) The source of the disease (reservoir)

(2) The means by which the disease may be transmitted (mode of transmission).

(3) A susceptible person (host).

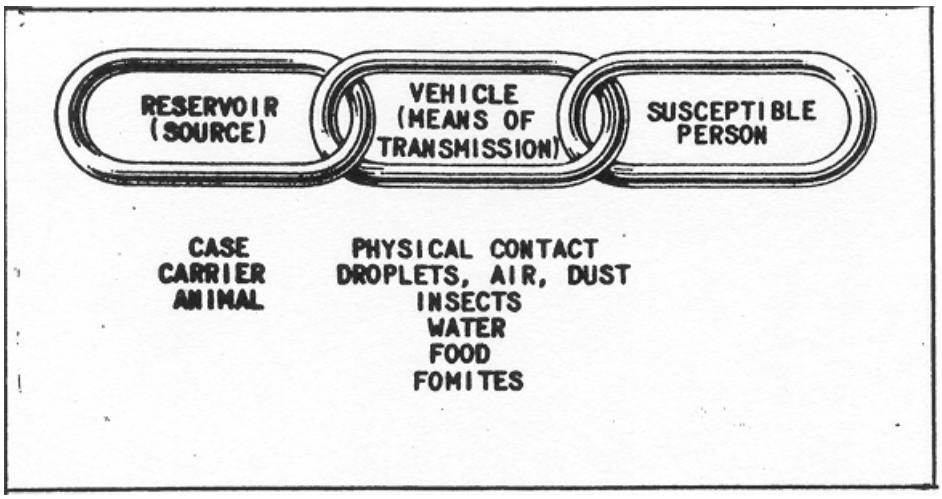


Figure 1-1. The chain of disease transmission

b. If anyone of the links in the chain is broken, the disease cannot spread.

c. Infection is the entry and multiplication of any infectious agent in the body. The period of time between infection and onset of signs and symptoms of disease is called the incubation period. However, infection does not always result in recognizable disease. Frequently the body has enough resistance or immunity to prevent disease development, in which event a carrier state results. A carrier can transmit disease in the same way as can a case (a person who is ill) with the same infection. Since the carrier is infected but is not sick, the carrier often goes undiscovered as a source of disease, or else is discovered only through very thorough clinical and laboratory effort. When an infectious agent strikes a community or military unit, its presence quite likely will be manifested variously in the affected individuals by all grades of severity, from the carrier state, through the mild disease states, the typical disease states, to the most severe and possibly fatal reactions. This broad gradation of manifestation of the infectious process in a group is called the spectrum of infection. Within this spectrum, there are always some cases that are so mild that they are not seen by the medical service, while others are entirely asymptomatic. These subclinical or asymptomatic infections, which are usually unreported, constitute what is commonly referred to as the "iceberg effect." They, like the portion of an iceberg that is under the water, are unseen; therefore, their danger is not fully realized by the public. In preventive medicine,

however, these cases are very important, because persons with subclinical or asymptomatic diseases can frequently transmit the diseases as efficiently as those persons manifesting all the symptoms.

1-6. DISEASE AGENTS

A disease-producing agent is any living organism or toxic substance (frequently produced by an organism) that may cause death, disease, or infection in another living organism.

a. Disease-producing infectious agents may be either chemical agents or infectious agents

(1) Chemical agents causing disease may be toxic metals or their oxides (lead, zinc, and so forth); poisonous chemicals such as pesticides (DDT, malathion, and so forth); or toxins produced by living organisms as a result of their metabolic processes (Clostridium botulinum, Staphylococcus aureus, and so forth.

(2) Disease agents may also be living organisms, such as viruses, rickettsia, bacteria, fungi, protozoa, and helminths (parasitic worms). These agents will be discussed in detail in Lesson 2.

b. There are several factors that affect the agent's ability to cause an infection:

(1) Pathogenicity--the ability to produce disease. This factor varies widely between the various categories of agents.

(2) Virulence--the agent's ability to overcome the resistance of the host.

(3) Resistance--the resistance of the agent itself to drying, disinfectants, and therapeutic drugs.

(4) Infectivity--the ability of the agent to penetrate, multiply, and produce change in the host.

1-7. SOURCES OF DISEASE (RESERVOIRS)

The source of disease may be a case, a carrier, or an animal.

a. **Case.** A person who is actually ill with a disease is called a case. A case is a common source of infection.

b. **Carrier**. A person who harbors disease organisms but who is not ill is called a carrier. This person can spread the germs in the same manner as the case. Actually, he is more dangerous because he may not know that he is harboring the infectious germs.

c. **Animal**. An animal can actually be ill with disease or it can harbor the organisms, spreading it to humans in either instance. The term animal means any member of the animal kingdom, thus including insects as well as mammals.

1-8. MEANS OF DISEASE TRANSMISSION (MODE OF TRANSMISSION)

a. **Physical (Direct) Contact.** Certain diseases are spread by physical contact with an infected person. Examples are syphilis, gonorrhea, and scabies.

b. **Indirect Contact**.

(1) Droplets, air, and dust. Droplets are vehicles by which a disease may be transmitted from an infected person to susceptible persons. When an infected person coughs, sneezes, or even talks, he spreads droplets containing disease germs. If other persons are close to the infected person, they may inhale some of these droplets. Furthermore, some germs expelled from the respiratory tract are extremely small and light in weight and may remain suspended in the air for hours or may be resuspended in dust. Inhalation of these germs by susceptible persons may also result in disease. Many of the respiratory diseases are transmitted in these ways.

(2) Fomites. Articles contaminated with disease germs from an infected person may become vehicles of disease transmission if a susceptible person uses them. Examples of fomites are contaminated clothing, bed linen, and eating utensils.

c. **Arthropods or Other Vectors.** Flies, fleas, mosquitoes, ticks, mites, and lice are among the arthropods that spread disease from person to person or from animal to person. Insects are involved in both the direct and the indirect transmission of disease. A mosquito, for example, can pick up disease germs when it bites a person sick with a disease such as malaria. Later, when the mosquito bites another person, it injects the disease germs. The mosquito is, therefore, the vector by which the disease is transmitted from one person directly to another person. The fly, on the other hand, transmits disease germs indirectly. It can pick up disease germs on its body when it comes in contact with filth and may deposit these germs on food. If a person eats this food, he may become ill.

d. **Water and Food.** Certain disease germs are transmitted through the consumption of foods such as raw fish and improperly cooked meat and poultry. However, most of the diseases that are transmitted by food and water are the result of contamination of the food or water with feces or other infectious material from a person or animal. If water or food so contaminated is not properly treated, the germs therein may infect the consumer. Outbreaks of disease will occur when personal hygiene and proper sanitation practices applicable to food handling, water purification, water disposal, and the control of flies and other vermin are not properly observed and enforced. Among the diseases usually transmitted by contaminated food or water are typhoid fever, infectious hepatitis, cholera, dysentery, and food poisoning (due to staphylococci, Clostridium perfringens, and other organisms.)

1-9. SUSCEPTIBLE PERSON (HOST)

A susceptible or non-immune person is one who has little resistance against a particular organism and who, if exposed to this organism, is likely to contract disease. By contrast, an immune person is one who has a high degree of resistance to the organism and who, when exposed, does not develop the disease. Immunity to many diseases is relative and can be overcome by sufficient exposure to the diseases. The term host is used to connote the living body upon which or in which a disease agent or parasite lives--the final recipient of an infectious agent. The susceptibility of a host to invasion by infectious agents is dependent upon a number of factors.

a. **Age.** The very young and the very old are usually more susceptible to diseases than older children and young adults. In addition, certain diseases have a natural affinity for persons in certain age groups. Examples of these diseases are measles, chickenpox, mumps, and other diseases normally associated with childhood and adolescence.

b. **Physical Condition.** Persons who are in a state of malnutrition, suffering from extreme fatigue or exposure to the elements, or suffering from an imbalance in normal body functions have weakened resistance to the invasion of pathogenic organisms.

c. **Immunity.** The natural immunity acquired by an individual by virtue of having been infected by, or exposed to, an infectious organism and having developed antibodies against the agent reduces the likelihood of further or repeated infection. Similarly, one who has been artificially immunized against a disease will be similarly protected.

d. **Habits and Customs.** Local mores--particularly those found in undeveloped and underdeveloped regions--may be the cause of increased susceptibility to disease. Although these conditions are more appropriately discussed under sanitary practices than as host factors, their longstanding acceptance by certain cultures makes them inseparable from the host. Examples of these practices are the custom of rubbing the umbilical cord with dirt (in some South American cultures) and of defecating directly into rice paddies (as in Vietnam and other Asian cultures).

e. **Other Factors**. A number of other factors tend to predispose an individual to various diseases--not necessarily communicable diseases, but infirmities in general. Among these factors are race (hypertensive heart disease and sickle cell anemia in blacks); sex (breast cancer in women); occupation (lung cancer in asbestos workers); geographical location; socioeconomic status; and others.

1-10. MULTIPLE CAUSATION

The concept of multiple causation is analogous to that of the chain of disease transmission. Just as there are three links in the chain of disease transmission, there are also multiple causes of diseases rather than one simple cause for each disease. The various factors of host vulnerability, agent characteristics, mode of transmission, and reservoirs of a disease introduce a number of possibilities to which the cause of a disease may be attributed. An exaggerated illustrative example of the multiple causation concepts is the case of a pedestrian who was hit by an automobile and was taken to a hospital, where he subsequently died. In the autopsy, the pathologist attributed death to a ruptured spleen. The surgeon, however, considered the cause of death to be slow diagnosis in the emergency room, since the spleen could have been repaired had the diagnosis been made promptly enough. To the internist, the cause of death was shock. The investigating policeman blamed the death on excessive speed on the part of the driver, while the driver claimed that the pedestrian was intoxicated and reacted improperly. Which diagnosis is correct? In all probability, there is some merit in each of the arguments. Here we see a typical case of multiple causation where each of the factors involved could have been the cause of death, and each contributed to the death. Conversely, if the proper action had been taken to prevent the occurrence of each of the events, death could have been prevented. Just as there were several opportunities to prevent death in the example cited here, the chain of disease transmission presents several opportunities to interrupt the continuity that is required in the transmission of a communicable disease.

1-11. COMMUNICABLE DISEASE CONTROL

Disease control measures may be viewed as a means of breaking the links to the chain of disease transmission (figure 1-2). If anyone of the links in the chain is broken, disease will not occur. The infection chain for each specific disease usually has one link that is more vulnerable or more easily broken than the others. While the chain may be attacked at several points and all three links, the major effort usually is made against the weakest link. The one disease control measure that is applicable to all three links is personal hygiene. Personal hygiene is defined as the application, by the individual, of the principles of healthful living. It embraces more than mere personal cleanliness. Achieving a high level of personal hygiene requires the individual to practice health rules to safeguard his own health and the health of others. Every individual is potentially a source of disease, a vehicle of transmission, and a person susceptible (to a greater or lesser degree) to disease. Therefore, universal and unrelenting application of the principles of healthful living will do much to prevent the spread of disease.

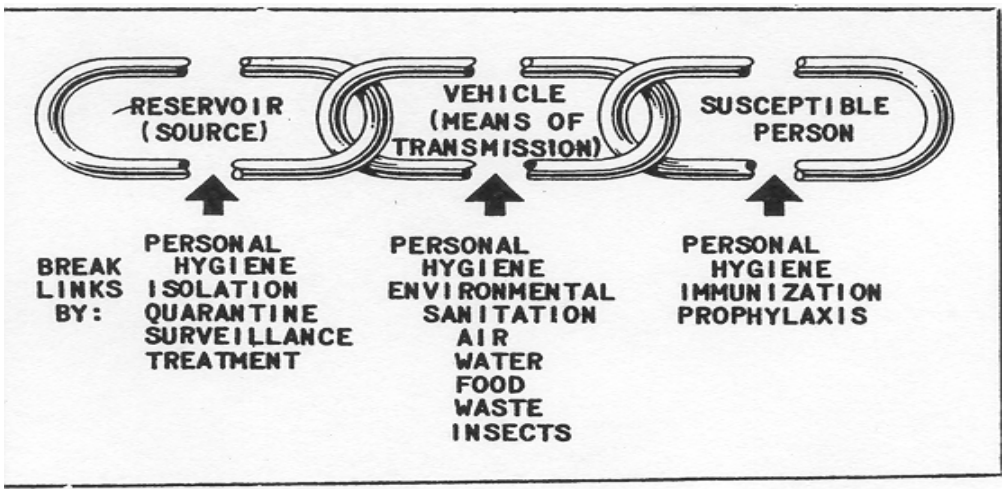


Figure 1-2. Break the chain of disease transmission.

a. **Control Measures Applicable to the Source**.

(1) Isolation is the separation of infected persons from others during the period in which the infection is communicable. This separation may be accomplished by having the sick person admitted to hospital isolation or by self-isolation practiced by the infected person. In self-isolation the infected person should separate himself from susceptible by a distance of more than 5 feet, practice good personal hygiene, and avoid the spreading of disease agents as much as possible.

(2) Quarantine is the restriction of movement of a well person who has been exposed to a communicable disease. The purpose of quarantine is to prevent contact between a probable carrier and other people who have not been exposed.

(3) Medical surveillance consists of observing and supervising someone who, through association with a disease source, may have been exposed to a disease.

Surveillance of these contacts permits early recognition of disease without restriction of movement. In the presence of a threatened epidemic, examination of all troops may be ordered.

(4) Prompt and adequate treatment of disease sources assists in the destruction of the infectious agent and subsequent reduction in the reservoir of disease agents.

b. **Control Methods Applicable to the Mode of Transmission**. Air, food, water, clothing, bedding, waste, people, and various forms of animal life in the environment have the capacity to transmit communicable disease agents. Environmental sanitation is essential for the control of transmitting agents. The following principles are included in environmental sanitation:

(1) Good personal hygiene by each individual.

(2) Avoidance of overcrowding and close physical contact.

(3) Proper ventilation of living quarters.

(4) Water purification.

(5) Careful selection and preparation of food.

(6) Maintenance of food service sanitation.

(7) Sanitary waste disposal.

(8) Proper control of disease-carrying insects and animals.

c. **Control Methods Applicable to the Susceptible Person**. A "susceptible" or "non-immune" is a person who has little resistance against a particular disease organism and who, if exposed to this organism, is liable to contract the disease. Protection of the susceptible requires use of all measures necessary to maintain or improve general health. The individual who has good mental and physical health has good resistance to disease. Good personal hygiene, including avoidance of known or suspected sources of disease, helps maintain health. A number of immunizing agents are available for use in conjunction with other measures for the control of some, but not all, communicable diseases. In some instances, suppressive drugs are available to decrease the severity of disease.

1-12. EPIDEMIOLOGY

a. Epidemiology is the study of the distribution and determinants of disease in human populations. It is the basic science of preventive medicine. Epidemiology is to preventive medicine what mathematics is to chemistry or physics; it is the practical tool used to study disease problems in the community. The distribution and determinants of a disease can be expressed by answering four questions.

(1) Who has the disease? In other words, which populations, or groups of persons within a given population, have high or low rates of a disease?

(2) Where is the disease? More specifically, in what regions of the city, the country, or the world is the disease most prevalent and most highly concentrated?

(3) How is the disease transmitted? Is it spread by personal contact, by means of an arthropod vector, via food and water, or by some other means?

(4) When does the disease occur? Is it endemic, existing at fairly constant levels? Is it epidemic, flaring up at certain times of the year? What is the incubation time? What is the normal course of the disease in terms of its duration?

b. An example of the epidemiology of a disease is that of syphilis. If we were to examine a textbook of medicine and look up the epidemiology of syphilis, we should very likely find a description similar to the following.

(1) First, who gets syphilis? We would find that the disease is most prevalent in the younger, most sexually active group of adults--chiefly in the 20-25 year age group. It is not as common among teenagers as is commonly believed. Syphilis is much more commonly reported by males than by females.

(2) Second, where do we find the disease? We learn that syphilis is a disease of large cities. It has a much lower incidence in rural areas. High rates are found in the United States, the Scandinavian countries, Western Europe, and Japan.

(3) Third, how is syphilis transmitted? Primarily by sexual intercourse, although congenital syphilis may occur occasionally in infants born of syphilitic mothers. In rare instances, the disease may be contracted by kissing. Cases acquired by intra-rectal intercourse and oral-genital contacts are not infrequent.

(4) Fourth, when does syphilis manifest its symptoms? We find that syphilis, compared to most infectious diseases, has a fairly long incubation period. Characteristically, the first symptoms appear in about three weeks, followed (in the untreated state) by secondary manifestations after about four to six weeks. These manifestations disappear spontaneously, even in the absence of treatment; however, they may be followed--after a period of from 5 to 20 years of latency--by explosively destructive lesions of late syphilis attacking the skin, bone, mucosal surfaces, or central nervous system.

c. From the information obtained in a situation such as that illustrated above, we are able to plan a preventive medicine effort toward eradicating (or at least minimizing the effects of) a disease. We know which groups we are dealing with, where the problem occurs, the means by which the disease is spread (and therefore the means by which to prevent its spread), and the critical periods of time during which we must act to obtain optimum benefit from our efforts.

1-13. USES OF EPIDEMIOLOGY

In general, epidemiology has three principal uses in the field of preventive medicine.

a. First, it may provide the key to discovering the etiology, or causative origin, of a disease whose exact cause is not yet known. For example, during the London cholera epidemic of 1849-54, John Snow, an English physician, established that cholera was caused by polluted water and not by "bad air" as most people at that time believed to be the cause. His hypothesis was based upon a study of the occurrence of the disease within the city and the source from which each affected family obtained its drinking water.

b. Second, epidemiology is used to investigate epidemics of diseases in which the causative agents are known, but the means of transmission are unknown. An epidemic of infectious hepatitis at Holy Cross University in 1968 was traced to a polluted water supply by means of the tools of epidemiology.

c. Third, epidemiology is a useful administrative tool in the implementation of a preventive medicine program. It assists us in planning the type of health care facilities needed, planning education programs, identifying high risk groups, identifying areas in which to locate health care facilities, and in many other facets of preventive medicine.

Continue with Exercises

EXERCISES, LESSON 1

INSTRUCTIONS. The following exercises are to be answered by marking the lettered response that best answers the question or best completes the sentence or by writing the answer in the space provided.

After you have completed all the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers.

1. In every major combat action in which the United States has participated, the leading cause of ineffectiveness has been:

   a. Shell shock and other neuroses

   b. Venereal disease.

   c. Fragmentation wounds.

   d. Poor leadership.

   e. Disease and non-combat injury.

   f. AWOL and desertion.

2. The health of a military command is the responsibility of the:

   a. Commander.

   b. Surgeon.

   c. Unit aidman.

   d. Physician assistant.

   e. Preventive medicine specialist.

3. Of the following listed terms, several may apply to an individual at one time or another. Which term is not used interchangeably with one of the others?

   a. Reservoir.

   b. Agent

   c. Host.

   d. Case.

   e. Carrier.

4. Malaria is a type of:

   a. Intestinal disease.

   b. Respiratory disease.

   c. Venereal disease.

   d. Arthropod-borne disease.

5. Which of the following are links in the chain of disease transmission? (More than one response is correct.)

   a. Susceptible.

   b. Epidemiology.

   c. Mode of transmission.

   d. Reservoir.

   e. Isolation.

   f. Personal hygiene.

6. Carriers of communicable diseases and persons with sub-clinical or asymptomatic cases of diseases constitute a phenomenon known as the _____.

7. Disease-producing agents may be either _____ or _____ agents.

8. Match each of the terms in Column I with an appropriate definition listed in Column II.

Column I	Column II
a. ____Pathogenicity	(1) Ability to overcome the resistance of a susceptible.
b. ____Virulence	(2) Capacity for reproduction in air as well as in the absence of air.
c. ____Resistance	(3) Ability to withstand the action of disinfectant or antibiotic.
d. ____Infectivity	(4) Ability to cause a disease.
	(5) Vulnerability to detection by laboratory methods.
	(6) Ability to invade a susceptible multiply, and effect a change.

9. The source (reservoir) of a disease may be a _____, _____, or _____.

10. Means of disease transmission include: _____, _____, _____, _____, and _____.

11. Animals (such as rodents) and arthropods, which live on such animals and which are capable of transmitting diseases to humans are referred to as disease _____.

12. The final recipient of a disease agent is known as the _____.

13. The fact several factors, each of which may constitute a link in the chain of disease transmission, may occur at the same time is known as the concept of _____.

14. Which link in the chain of disease transmission is best broken by:

    a. Food service sanitation? _____.

    b. Isolation? _____.

    c. Personal hygiene? _____.

    d. Quarantine? _____.

    e. Immunization? _____.

    f. Proper ventilation? _____.

15. The study of the distribution and dynamics of disease in human populations is known as _____. This study may be expressed in simplified terms by four questions:

    a. _____

    b. _____

    c. _____

    d. _____

16. The three general uses of the science of epidemiology are:

    a. To discover the _____ of a disease.

    b. To discover how a disease of known origin is _____.

    c. As an _____.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 1

1. e (para 1-1)

2. a (para 1-2)

3. b (para 1-3)

4. d (para 1-4d)

5. a, c, d (para 1-5a)

6. "Iceberg effect" (para 1-5c)

7. Chemical, infectious (para 1-6a)

8. a. (4) (para 1-6b(1))

   b. (1) (para 1-6b(2))

   c. (3) (para 1-6b(3))

   d. (6) (para 1-6b(4))

9. Case, carrier, animal (para 1-7)

10. Physical contact (para l-8b)
    Droplets (air, dust)
    Arthropods
    Water (food)
    Fomites

11. Vectors (para 1-3g)

12. Host (paras 1-3h, 1-9)

13. Multiple causation (para 1-10)

14. a. Vehicle (para 1-11)
    b. Reservoir (source)
    c. All three
    d. Reservoir (source)
    e. Susceptible (host)
    f. Mode of transmission

15. Epidemiology (para 1-12)
    a. Who (has the disease?)
    b. Where (is the disease?)
    c. How (is the disease transmitted?)
    d. When (does the disease occur?)

16. a. Cause (etiology) (para 1-13)
    b. Transmitted
    c. Administrative tool

End of Lesson 1

LESSON ASSIGNMENT

LESSON 2	Public Health Microbiology.
TEXT ASSIGNMENT	Paragraphs 2-1 through 2-28.
LESSON OBJECTIVES	After completing this lesson, you should be able to:

2-1. Identify the types of microorganisms that are important in public health.

2-2. Identify specific characteristics that enable the microbiologist to distinguish between various Microorganisms.

2-3. Identify helminthic parasites that are of public health importance.

SUGGESTION After completing the assignment, complete the exercises of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 2

PUBLIC HEALTH MICROBIOLOGY

SECTION I. INTRODUCTION

2-1. GENERAL

a. **Microbiology**. Microbiology is the study of microscopic forms of life (microbes). It includes their identification, their occurrence in nature, their physiology and reproduction, the role they play in nature and in industry, their relationship to one another and to man, and the means of controlling their activities. Microorganisms are tiny, simple forms of life that can be seen only through a light microscope or an electron microscope. Some microbes are complete as single cells, whereas others are multicellular. They include the following groups of organisms, each of which will be discussed in one of the subsequent sections of this lesson:

(1) Bacteria (plural of bacterium).

(2) Viruses (plural of virus).

(3) Fungi (plural of fungus).

(4) Protozoa (plural of protozoan).

(5) Certain helminths.

b. **Importance of Microbes**. The importance of microorganisms is little appreciated by the average person in civilized society. We take for granted the many tremendously important functions performed by microbes in both nature and industry. The decomposition of organic matter-dead animals and plants-into simple inorganic compounds that serve as nutrients for plant life is accomplished by microbes. The action of microorganisms contributes to the clarification and purification of polluted waters. In the food and beverage industries, microbes are harnessed in the production of buttermilk, cheese, beer, wine, and other products. At the same time, they pose a threat, in that certain type, if not controlled, have properties that lead to the destruction of such products. Microbes also play a role in the drug, tobacco, oil, lumber, and clothing industries.

c. **Pathogenic Organisms**. Aside from the beneficial role played by microbes in maintaining the life cycle in nature, some of them have harmful effects upon other forms of life--plants, animals, and man. Of the billions of microorganisms existing in nature, only a relatively few are pathogenic (that is, disease producing) to man. However, it is with these relatively few pathogenic organisms that we are concerned in public health microbiology.

2-2. TYPES OF CELLS

a. **General**. The cell is the basic unit, or building block, of all living matter--plant or animal. A microorganism may consist of a single cell (unicellular) or, as in the higher forms of life, it may consist of a number of cells arranged to perform specific functions (multicellular). The cell determines not only the structure of organism, but also its function. The cell itself is made up of components that have specific functions, just as the organs comprising the body have specific functions. Modern classification systems are based largely on differences in cell types. The more primitive cells are known as prokaryotes, whereas higher forms are known as eucaryotes. Certain microorganisms exhibit prokaryotic cell types while other microbes are of the eucaryotic cell type.

b. **Eucaryotic Cell Components**. Eucaryotic cell organization is common to fungi and protozoa as well as to all higher multicellular organisms, including humans. Since every organism has a distinctive type of cell, there is no such thing as a "typical" cell. However, most eucaryotic cells consist of the following parts (figure 2-1).

(1) Cytoplasm. The cell is filled with a jelly-like fluid called the cytoplasm. Proteins and dissolved nutrients that are suspended in this fluid are used by the cell for metabolism and to build new cell structures. The cytoplasm contains organelles, intracellular structures bounded by membranes that separate their contents from the cytoplasm. Some organelles are described below.

(2) Nucleus. The organelle that contains the genetic material (Chromosomes) and nucleolus.

(3) Genetic material, chromosomes. The genetic material contains the hereditary characteristics of the cell. It is made up op several long strands of DNA (deoxyribonucleic acid) that are called chromosomes.

(4) Nucleolus. An area of the nucleus that manufactures structural components used in protein synthesis.

(5) Nuclear membrane. Separates the nucleus from the cytoplasm, although pores permit the entrance and exit of certain chemicals.

(6) Mitochondria. Site where metabolic enzymes perform respiration (chemical oxidation) within the cell (conversion of food energy.)

(7) Cell membrane. Encloses the cell and governs the exchange of food material and release of waste products between the cell and its surroundings. In certain types of organisms, the cell membrane is enclosed within a cell wall that provides strength and rigidity, and is common to all classes of microorganisms.

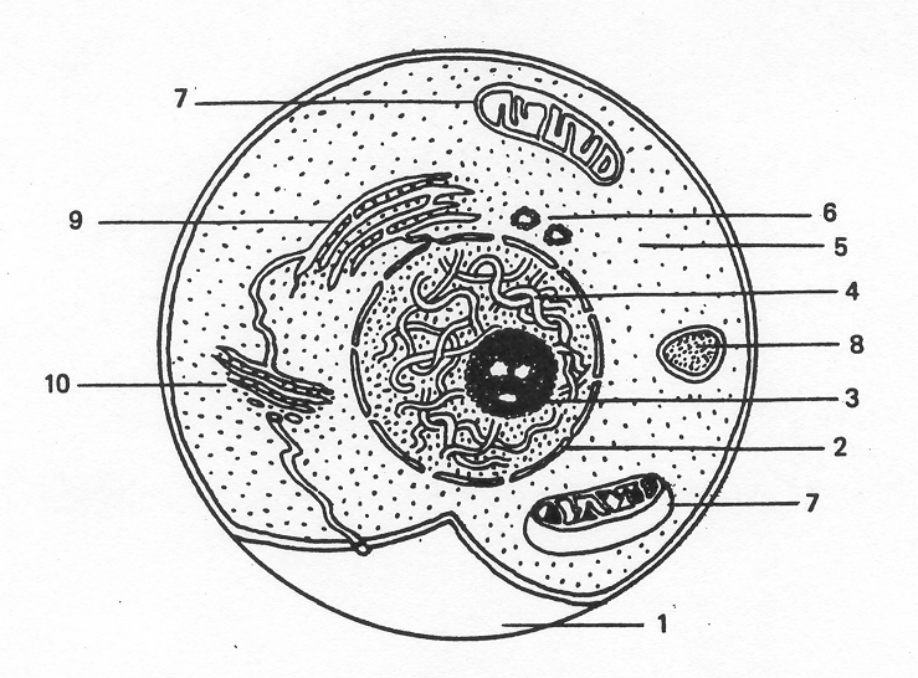


Figure 2-1. The eucaryotic

(8) Lysosomes. Contains digestive enzymes that break down large molecules into smaller nutrients that can be oxidized by the mitochondria.

(9) Endoplasmic reticulum. Contains many ribosome's that synthesize enzymes and other proteins

(10) Golgi apparatus. Accumulates proteins and enzymes and conveys some of them to the cell membrane for secretion. Some of the other enzymes are encased in lysosomes.

c. **Prokaryotic Cell Components**. Although structurally different and less complex than the eucaryotic cells, procaryotes (figure 2-2) still perform most of the same functions. The nuclear genetic material (DNA) consists of a single, circular, threadlike chromosome. It is not enclosed within a nuclear membrane, but is distributed in masses throughout the cytoplasm. Another striking difference between the eucaryotic and prokaryotic cells is that the cytoplasm of procaryotes does not contain membrane-enclosed bodies such as mitochondria, endoplasmic reticulum, or golgi apparatus. Some procaryotes possess mesosomes that are somewhat similar to mitochondria in their function. Ribosomes (sites of protein synthesis) are distributed throughout the cytoplasm. With few exceptions, the prokaryotic cell is surrounded by a cell wall. These cell walls are not made of cellulose or polysaccharides; but contain peptidoglycan, which is found only in procaryotes. Cell division is by binary fission rather than mitosis or meiosis.

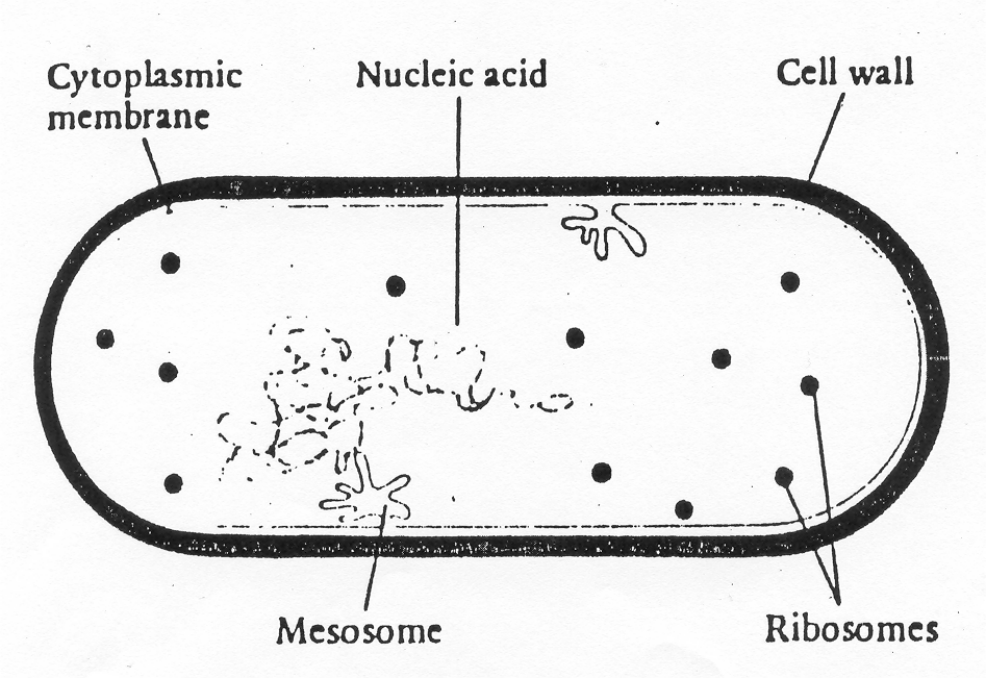


Figure 2-2. Prokaryotic cell.

d. **Class Sporozoa (Obligate Parasites)**. These organisms are parasites having no organelles of locomotion. They have a complicated sexual-asexual life cycle consisting of several morphological stages. In order for the entire cycle to be completed, an intermediate arthropod host is necessary. These parasites invade and multiply in the cells of the host. The malaria parasites of man belong to this group. Figure 2-14 illustrates the life cycle of the malarial parasite.

2-3. TYPES OF CELL REPRODUCTION

a. **General**. Growth and reproduction are accomplished by means of cell division. One of the characteristics of cells is their ability to reproduce their own kind of division. This does not mean, of course, that the cells of a mouse will divide and produce another mouse. It does mean, however, that tissue cells in a certain part of a young mouse will divide to form additional cells of the same type, thereby producing growth in the organ made up of that particular tissue. In the case of microscopic, unicellular organisms, cell division produces a new organism.

b. **Mitosis**. Mitosis is a complicated form of cell division found primarily in the higher forms of life, but which may occur in some of the lower forms, such as certain protozoa. In mitosis (figure 2-3), the chromatin becomes organized into pairs of chromosomes and the centrioles (comprising the centrosome) begin to move toward opposite poles, forming a spindle. The nuclear membrane and nucleolus break down, the centrioles reach opposite poles, and the pairs of chromosomes separate, moving along the spindle toward the poles. After reaching the poles, the chromosomes consolidate each centriole forms a new centriole, two new nucleoli, and nuclear

membranes are formed. When cell division is complete, each new cell is ready to undergo the same process.

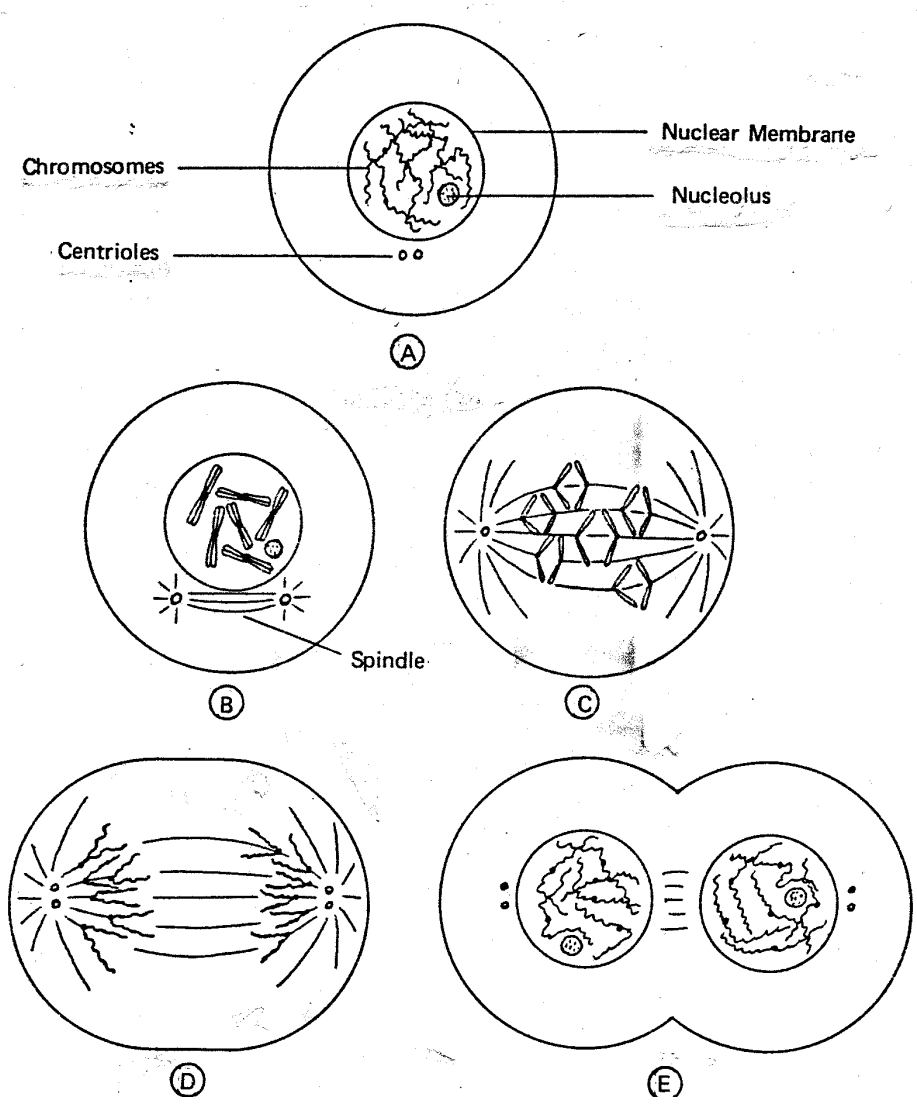


Figure 2-3. Types of cell reproduction-mitosis.

c. **Meiosis**. Meiosis is similar to mitosis, but the chromosome pairs do not separate as in figure 2-3. Instead, half of the chromosomes go to each centriole, resulting in two new cells having half the number of chromosomes as the parent cell. This type of cell division is found only in the sexual cells of the higher forms of life. The cells formed by meiosis are known as gametes. When two gametes of opposite sexes of the same species combine, they form a zygote, which contains the same number of chromosomes as each parent cell and half of the hereditary characteristics of each.

d. **Amitosis**. Amitosis is a simple type of cell division in which the nucleus undergoes cleavage, followed by that of the cytoplasm, without the formation of a

spindle and the alignment and polarization of chromosomes (figure 2-4). Each cell receives a copy of the parent chromosomes. Amitosis is rare in the higher forms of life, but it may occur in the lower forms.

Figure 2-4. Types of cell reproduction-binary fission.

e. **Binary Fission (Transverse Fission)**. Binary fission is the simplest form of cell division and that most frequently seen in the study of microbiology. Binary fission normally occurs when a cell reaches an optimum size and environmental conditions are favorable. The center of the cell constricts, a new cell membrane (or cell wall) forms between the two portions of the cell, and cleavage occurs, forming two new cells. A copy of the chromosome is produced so that each new cell has the same genetic information. Figure 2-4 is a graphical representation of binary fission.

2-4. CLASSIFICATION OF ORGANISMS

a. **General**. Classification is an orderly arrangement of organisms with similar physical, biochemical, and genetic characteristics into groups. Taxonomy, the science of classification, is subject to change as new and more precise information becomes available.

b. **Five Kingdom System**. The five-kingdom system is based on the difference among eucaryotic and prokaryotic forms of life, including their cellular organization (unicellular, unicellular-colonial, or multicellular) and their nutrition (absorptive, ingestive, photosynthetic, or combination of these). The characteristics of the kingdoms are shown in Table 2-1. You will notice that viruses are not included in any kingdom, that is because they are not cells nor are they living organisms.

c. **Scientific Names of Organisms**. Every living organism is assigned a scientific name (usually in Latin) by which it is identified uniformly by scientists worldwide. The scientific name of an organism, which is always italicized, consists of two identifiers-the genus and the species. The first letter of the genus is capitalized. For example, b*os taurus* denotes a member of the genus Bas (cattle), and *taurus* indicates the species *taurus* (common European cattle). It is customary, when discussing additional species of a genus previously mentioned, to indicate the genus by the first letter of the genus name. For example, *B. indicus* refers to Indian, or Zebu (Brahman) cattle. When referring collectively to a number of species within the same genus, a writer frequently uses the genus name, followed by the abbreviation "spp" in lower case letters. For example, *Salmonella* spp refers collectively to species of bacteria within the genus *Salmonella*.

Kingdom	Cellular Organization	Cell Wall	Mode or Type of Nutrition	Microbial Representative
Plantae	Eucaryotic and Multicellular	Present	Primarily Photo-synthetic	None
Animalia	Eucaryotic and Multicellular	Absent	Ingestive and some absorptive	None
Protista	Eucaryotic, Unicellular, and some colonial forms	Absent except with algae	Absorptive, ingestive, photo-synthetic, and combinations	Protozoa, Algae
Fungi	Eucaryotic, multicellular and unicellular	Present	Absorptive	Molds and Yeast
Monera or Pro-karyotae	Prokaryotic and unicellular	Present	Absorptive	Bacteria

Table 2-1. Characteristics of the five kingdoms.

2-5. OBSERVATION AND MEASUREMENT

a. **Observation**. Although microbes are considered to be among the first living things on earth, they were not actually seen until 1675. In this year Anton van Leeuwenhoek of Holland, the inventor of the microscope, first described protozoa as viewed under his instrument. His better microscopes had a magnification of about 160. The standard light microscopes of today magnify an object up to about 1000 diameters. The electron microscope that differs from the ordinary light microscope by focusing an indirect image of the specimen on a screen will provide a magnification of 200,000 times. By means of extremely precise photographic methods, we can achieve a further enlargement of 10 times. This gives us a theoretical capability of examining specimens with a magnification of two million.

b. **Units of Measurement**. A special scale can be used on a light microscope to enable measurement of microorganisms. Objects less than about 0.3 microns cannot be seen when using a light microscope. The following units of measure are normally used in the measurement of microscopic objects:

(1) Micron (μ): 1/1000 millimeter (about 1/25400th of an inch).

(2) Millimicron (mμ): 1/1000μ (1 billionth of a meter, or 1 millionth of a millimeter.

Section II. BACTERIA

2-6. GENERAL

Bacteria are microscopic, unicellular organisms of the Kingdom Monera. They are widely distributed in nature, occurring nearly everywhere. They are found on and in our bodies, in the air we breathe, in the food we eat, and in the water we drink. They are part of the natural flora of the skin, naso-pharyngeal mucosa, and alimentary tract. They are particularly plentiful in the upper layers of the soil, playing an important part in the decomposition of organic matter and in nitrogen fixation. There are several thousand known species of bacteria; however, of this number, only about 100 species are pathogenic to man. Some also produce disease in the lower animals. The vast majority of bacteria, however, do not attack man or beast. They are not only harmless, but they are quite beneficial; without their help, all plant and animal life would cease. In this section, however, we are concerned with those bacteria that cause disease in man. Bacteria produce disease in man by invading the body and reproducing (colonizing), thereby causing damage to the host cells. Reproduction is by binary fission (paragraph 2-3e). Under favorable conditions, bacteria reproduce very rapidly. Some newly formed bacteria can mature and divide to form two new bacteria every 20 to 30 minutes. Most bacteria have independent metabolism, enabling them to live and multiply in a favorable medium outside of a host cell.

2-7. MORPHOLOGY OF BACTERIA

Bacteria range in size from 0.15μ (microns) in diameter for some of the smaller, pathogenic cocci, to 20μ in length for the larger, nonpathogenic bacilli. The three principal shapes of bacteria are shown in figure 2-5.

a. **Coccus (Plural, Cocci)**. Spherical bacteria occurring in any of the following arrangements:

(1) Singly (coccus). Figure 2-5 A.

(2) In pairs (diplococcus). Figure 2-5 B.

(3) In chains (streptococcus). Figure 2-5 C.

(4) In clusters (staphyloccus) Figure 2-5 D.

(5) In clusters of four (tetrad) or eight (cube). Figure2-5 E.

b. **Bacillus (Plural, Bacilli)**. Rod-shaped bacteria occurring in any of the following arrangements:

(1) Singly (bacillus). Figure 2-5 F.

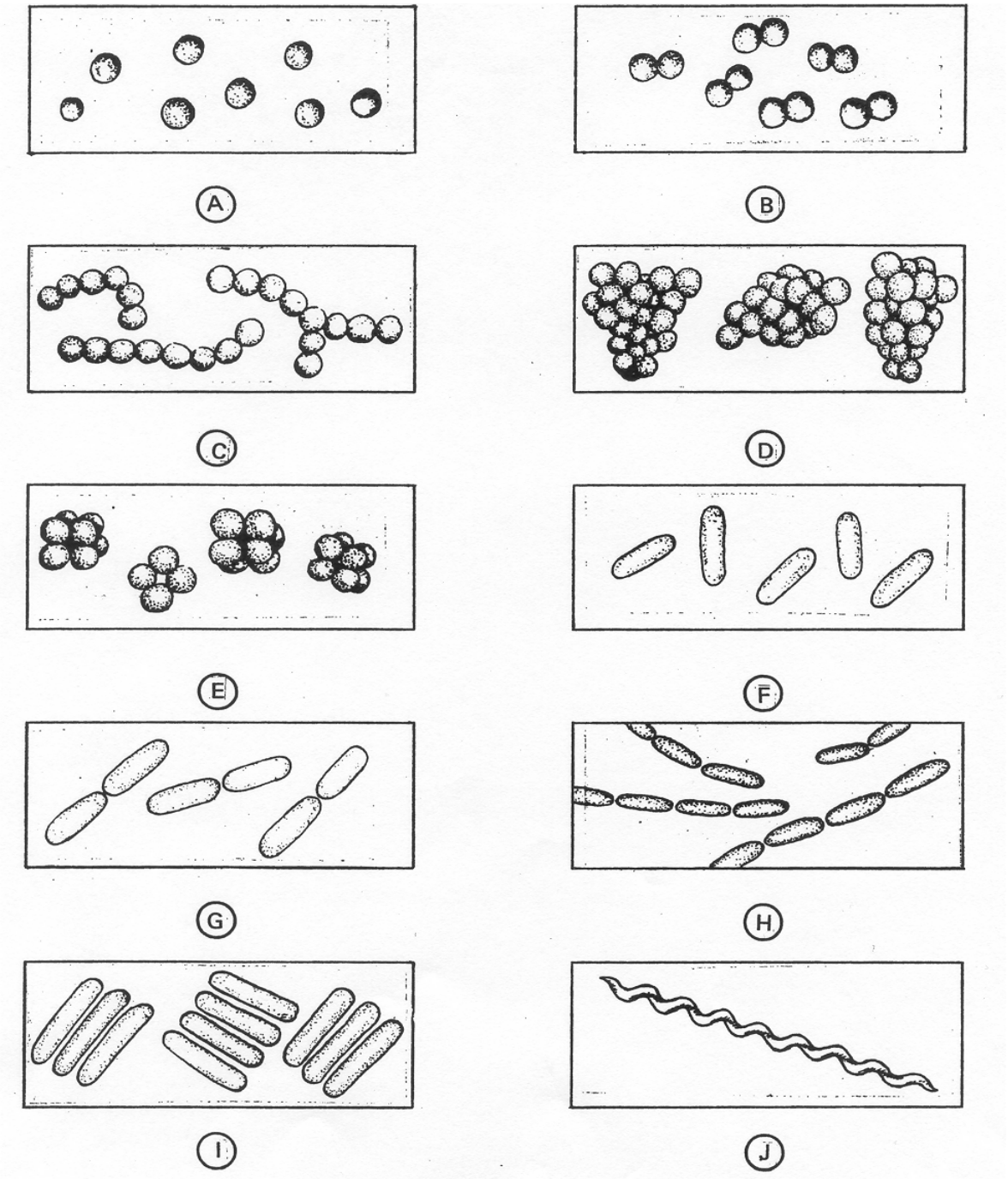


Figure 2-5. Shapes of bacteria

(2) In pairs (diplobacillus). Figure 2-5 G.

(3) In chains (streptobacillus). Figure 2-5H.

(4) In palisades (palisade). Figure 2-5I.

c. **Spirals.**

(1) Spirillum (plural, Spirilla)--spiral, corkscrew-shaped organisms whose long axes remain rigid while in motion.

(2) Spixochete--a spiral microorganism whose long axis flexes when it is in motion. Figure 2-5 J.

d. **Other Variations of Shapes.**

(1) Coccobacillus. A short, plump bacillus with rounded ends resembling a coccus in shape and arrangement.

(2) Vibrio. A curved bacillus.

2-8. STRUCTURE OF BACTERIA

The typical structure of a bacterial cell is shown in figure 2-6. It consists of the following general structures, found in all bacterial cells; and special structures, found in specific types of bacterial cells:

GENERAL STRUCTURES SPECIAL STRUCTURES

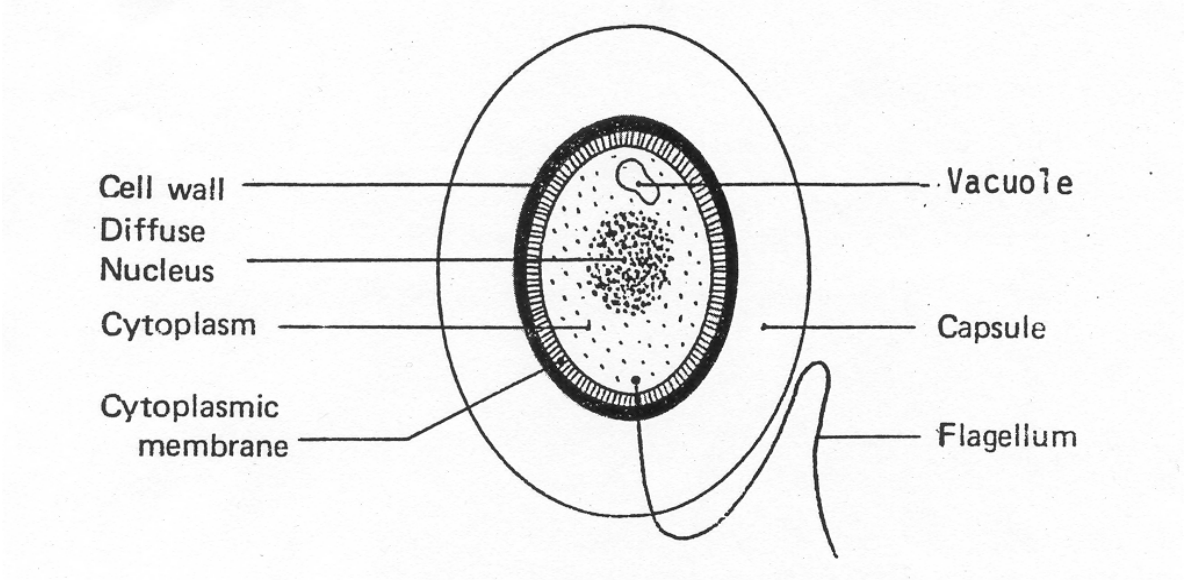


Figure 2-6. Bacterial cell structure.

a. **General Structures.**

(1) Cell wall. A cell wall is a layer of lipid and protein that encloses the protoplasm of the cell giving rigidity to the bacterial shape.

(2) Cytoplasmic membrane. A cytoplasmic membrane is a thin semi permeable membrane located directly beneath the cell wall, governing osmotic activity.

(3) Cytoplasm. The cytoplasm is the protoplasmic or vital colloidal material of a cell; the site of metabolic activities.

(4) Genetic material. Genetic material is a circular, single-stranded DNA diffused throughout the cytoplasm. It carries the genetic code of heritable traits and is responsible for directing metabolism and replication of the cell. The bacterial cell does not have a nuclear membrane or a well-defined nucleus.

b. **Special Structures**.

(1) Capsule. A capsule is relatively thick layer of mucoid polysaccharides (slime layer) around a bacterium. A capsule serves as a defense mechanism.

(2) Flagellum. A flagellum is a protoplasmic strand of elastic protein originating in the cytoplasmic membrane, and extending from the body of the cell. A flagellum serves as an organ of locomotion. The arrangement of flagella (plural) is peculiar to the species.

(3) Spore. A spore is a metabolically resistant body formed by a vegetative bacterium, which is in a dormant state, to withstand an unfavorable environment. Only bacilli form spores. The position and size of a spore within a bacillus is peculiar to the species. Bacteria that are actively reproducing by fission do not produce spores (sporulate).

(4) Inclusion bodies. Inclusion bodies are vacuoles of reserve or waste materials contained within the cytoplasm.

(5) Ribosomes. Ribosomes is a site of protein production.

2-9. IDENTIFICATION OF BACTERIA

Since there are several thousand species of bacteria, it would be impossible to identify them on the basis of appearance alone. Therefore, the bacteriologist employs a wide variety of techniques, based upon known characteristics of specific bacteria, to arrive at the identity of a given specimen. The following characteristics, which are used frequently as terms of reference, assist the microbiologist in the positive identification of bacteria as well as in eliminating them from consideration.

a. **Food Requirements**.

(1) Natural media. In their natural habitat, bacteria that cause human disease are usually one of two types: saprophytes grow on dead organic matter and parasites grow on living tissue.

(2) Artificial media. In the laboratory, a few bacteria will not grow on any artificial culture medium. However, most bacteria will grow on culture medium that provides sources of carbon, nitrogen, minerals, water, and energy. Some bacteria grow only on special culture media that provides certain amino acids, vitamins, serum, or other special ingredients. One of the most common media used in a clinical laboratory contains the ingredients listed plus sheep blood. Various types of growth patterns and hemolysis can be observed from this growth.

b. **Oxygen Requirements**.

(1) Aerobes--grow in the presence of free oxygen.

(2) Anaerobes--grow only in the absence of free oxygen.

(3) Facultative aerobes (anaerobes)--able to adjust to an aerobic (anaerobic) environment.

(4) Microaerophiles--require small amount of free oxygen for growth.

c. **Types of Hemolysis**.

(1) Beta-hemolytic--can cause complete hemolysis (rupture) of red blood cells.

(2) Alpha hemolytic--causes a chemical change of hemoglobin in red blood cells.

(3) Gamma hemolytic--do not cause hemolysis.

d. **Staining Characteristics**. Specimens are normally stained prior to microscopic examination. Various species react differently to the stains. In the gram stain method, a specimen is stained with crystal or gention violet, followed by iodine. It is then washed with alcohol or acetone-ether and stained with safranin, a red or brown counter stain.

(1) Gram-positive--bacteria which, when stained by the gram stain method, retain the gram stain color (dark violet or purple).

(2) Gram-negative--bacteria which, when stained by the gram stain method, do not retain the gram stain color (dark violet or purple), but retain the color of the counter stain (red or pink).

(3) Acid-fast--bacteria that when stained with certain dyes and then treated with an acid, followed by a counter stain, retain the color of the dye.

(4) Nonacid-fast--bacteria that when treated as in (c), above, retain the counter stain rather than the dye.

e. **Microscopic Examination**.

(1) Shape (refer back to para 2-7).

(2) Arrangement (refer back to para 2-7).

(3) Size.

(4) Spore formation--spore formers or non-spore formers. (para 2-8b(3)).

(5) Motility--motile or nonmotile. (determined from an instained wet preparation).

f. **Pathogenicity**.

(1) Enzymes. Certain bacteria produce specific enzymes that enable the bacterial infection to invade the tissues of the host. Some of these enzymes break down cell membranes or chew up the chemicals that hold tissue together. The effect of this enzymatic action is to enlarge the site of infection.

(2) Production of toxins.

(a) Exotoxins--extremely potent proteins that are produced in bacterial cells and which diffuse freely into the cells of host tissues, causing severe systemic poisoning.

(b) Endotoxins--lipopolysaccharide chemicals, less potent than exotoxins, that are part of gram-negative bacterial cells and that affect the host cells only after the bacterial cell disintegrates.

2-10. PATHOGENIC BACTERIA

Table 2-2 presents a list of the principal pathogenic bacteria of public health importance, organized in such a way as to illustrate the means of identification

IDENTIFICATION GROUP	SPECIES	CAUSATIVE AGENT	REMARKS
GRAM-POSITIVE COCCI			
Alpha hemolytic	Streptococcus pneumonia	Lobar pneumonia, meningitis	Produces exotoxin causing skin rash
Beta hemolytic	Streptococcus pyogenes	Impetigo, septic sore throat, scarlet fever	Produces exotoxin causing food poisoning
	Staphylococcus aureus	Upper respiratory infections, boils, surgical infections, food poisoning, toxic shock syndrome	
GRAM-NEGATIVE COCCI	Neisseria gonorrhea	Gonorrhea. gonorrheal conjunctivitis	Kidney-shaped diplococcic
	Neisseria meningitidis	Epidemic cerebrospinal meningitis	Kidney-shaped diplococcic
GRAM-POSITIVE BACILLI Aerobic	Corynebacterium diphtheriae	Diphtheria	Produces powerful exotoxin causing inflammation of mucosa and impairment of vital organs
Aerobic. spore-forming	Bacillus anthracis	Anthrax (Chiefly in herbivorous animals,but also in man)	Forms a capsule
Aerobic. acid-fast	Mycobacterium tuberculosis	Tuberculosis (man)	
	M. bovis	Tuberculosis (cattle and man)	
	M. leprae	Leprosy (Hansen's Disease)	
Anaerobic, spore-forming	Clostridium botulinum	Food poisoning	Produces powerful, lethal exotoxin
	C. tetan1	Tetanus (lockjaw)	Produces powerful, lethal exotoxin
	C. perfringens	Gas gangrene, food poisoning	
GRAM-NEGATIVE BACILLI	Escherichia coli	Traveler's diarrhea, urinary tract infection	Many of these and other species are part of the normal flora of the adult intestinal tract, but pathogenic when introduced into other parts of the body
	Pseudomonas aeruginosa	Burn wound infection	
	Klebsiella pneumonia	Pneumonia	
	Yersinia enterocolitica	Diarrhea	
	Salmonella typhi	Typhoid fever	
	S. paratyphi	Paratyphoid fever	
	Salmonella enteritidis (many serotypes)	Acute gastroenteritis (Salmoneliosis-“food poisoning”)	
	Shigella dysenteriae'		Also produces a paralytic exotoxin
	Shigella spp	Bacillary dysentery	

Table 2-2. Pathogenic bacteria of public health importance. (continued)

IDENTIFICATION GROUP	SPECIES	CAUSATIVE AGENT	REMARKS
GRAM-NEGATIVE BACILLI (continued	Vibrio cholerae	Cholera	Exototoxin causes "rice water" stool
	Vibrio parahemolyticus	Diarrhea	Food poisoning from contaminated shellfish.
SMALL GRAM-NEGATIVE BACILL			
Non-motile, nonsporeforrning	Brucella abortus	Contagious abortion in animals; brucellosis	Occurs in cattle
	B. suis		0ccurs in swine
	B. melitensis	(undulant fever) in man	0ccurs in sheep
	Hemophilus influenzae	Pharyngitis. otitis. sinusitis, pneumonitis, or meningitis.	More common pathogen in children
	Bordetella pertussis	Whooping cough	Encapsulated Exotoxin
	H. ducreyi	Chancroid	
	Yersinia pestis	Plague	
	Francisesella tularensis	Tularemia	A typical bacteria
	Chamydia trachomatis	Urethritis. inclusion conjunctivitis. Trachoma. lymphogranuloma venereum	A typical bacteria
	C. psittaci	Psittacosis (parrot fever, ornithosis	A typical bacterium, requires living host cell
	Mycoplasma (Ureplasma)	Nongonococcal urethritis	A typical bacterium, no cell wall requires special culture
	Mycoplasma pneumonia	Primary, atypical pneumonia	A typical bacterium, no cell wall requires special culture
SPIROCHETES			
	Treponema pallidum	Syphilis	Does not stain with ordinary stain nor grow on artificial media
	Borrelia recurrent is	Relapsing fever	
	Leptospirosa ichterohemorr hagiae (also L. canicola, L. autumnalis, and L. pomona)	Leptospirosis (Weil's disease, infectious jaundice)	May be stained and cultured (chick embryo)

Table 2-2. Pathogenic bacteria of public health importance. (concluded)

discussed in paragraph 2-9. The student should bear in mind that bacteria may fall into several groups; therefore, the appearance of a species under a particular heading in Table 2-2 does not imply that it may not be appropriately shown under another heading.

2-11. RICKETTSIAE, CHLAMYDIAE, AND MYCOPLASMP

a. **General**. The rickettsiae, chlamydiae, and mycoplasmas are bacteria that are different from the typical bacteria discussed above. These organisms have unusual and more exacting growth requirements. Several of these organisms are significant pathogens.

b. **Rickettsia**.

(1) Morphology. Rickettsiae are cocco-bacillary to coccal in shape, varying in size from 0.3 p in length. They are plemorphic—that is, they have many shapes within the same species--and are found either singly, in pairs, or in chains. They are gram-negative and difficult to strain, but when properly stained they can be observed under the ordinary light microscope. The cell structure of the rickettsiae is similar to that of the typical bacterial cell, frequently exhibiting a capsule.

(2) Physiology. Physiologically, the intracellular rickettsiae resemble typical bacteria in some respects and viruses in others. Like typical bacteria, they multiply by binary fission. However, like viruses, they are obligate parasites—that is, they require a living cell for their existence and propagation. They differ from viruses in that they do contain some enzymes and demonstrate some independent metabolism.

(3) Pathogenic rickettsiae. The rickettsiae are transmitted from animal, animal to man, or man to man through the body of an intermediate arthropod host. The most common arthropod vectors are fleas, lice, ticks, and mites. The rickettsiae are nonpathogenic to the arthropod vectors and, because of their minute size, are capable of being transferred from a parent arthropod to the offspring through a process known as transovarian transmission. An exception is the louse, which dies within 8 to 10 days after infection with *Rickettsia prowazekii*. This process is particularly common in ticks and mites. The infectious organism passes through the reproductive system of the adult female into the eggs, and is thus transmitted to succeeding generations. The rickettsial diseases are characteristically manifested by high fevers and skin eruptions. Table 2-3 lists the most common rickettsial diseases, the causative agents, the reservoirs of infection, and the arthropod vectors responsible for their transmission.

c. **Chlamydia**. Chlamydiae are nonmotile, coccoid bacteria ranging in size from about 0.2µ to lµ. Except for possessing a thicker cell wall, their structure and composition are like the typical gram-negative bacteria. However, they exhibit a unique developmental cycle within the higher living cells they infect. They are obligate intracellular parasites that are associated with several human diseases (Table 2-3).

DISEASE	CAUSATIVE AGENT	RESERVOIR	ARTHROPOD VECTOR
Epidemic typhus (classical, Old World, European, or louse-borne typhus)	*Rickettsia prowazekii*	Man	Body louse feces (Pediculus humanus corporis) (rarely the head louse--Pediculus humanus capitus)
Endemic typhus (murine, New World, or fleaborne typhus)	*Rickettsia typhi*	Rats (less often, mice)	Rat flea bite (rat to man or rat) Rat louse (rat to rat)
Rocky Mountain spotted fever	*Rickettsia rickettsii*	Rabbits, small rodents, dogs, opossum, foxes	Tick bite (many species)
Rickettsialpox	*Rickettsia akari*	House mice	Mite bite (Alloder-manyssus sanguineus
Scrub typhus (tsutsugamushi disease.)	*Rickettsia tsutsugamushi*	Rodents	Mite bite (Trombicula akamushi, T. deliensis, T. pallida)
Q fever	*Coxiella burnetti (Rickettsia burnetii)*	Cattle, sheep, goats, rodents	None. Acquired by breathing contaminated air, drinking milk from infected animals, or by direct contact. .

Table 2-3. Common rickettsial diseases

d. **Mycoplasma**. Mycoplasmas are the smallest organisms known that are capable of growth and reproduction outside of living host cells. Because of their variations of shape, the actual size of the individual cells is variable. It is generally agreed that they range from about 0.12µ to 0.25µ in diameter. The major difference

between the mycoplasmas and typical bacterial cells is that mycoplasmas completely lack a cell wall; therefore, they assume a coccoid shape. Cell reproduction is more complex than typical bacteria. They vary in their growth requirements, but all can be grown on special artificial media. Mycoplasmas cause a number of infections (Table 2-3).

Section III. VIRUSES

2-12. GENERAL

Viruses are submicroscopic, obligate, filterable intracellular parasites. In other words, they are organisms that are too small to be seen with an ordinary light microscope and which are totally dependent upon a host cell for survival. They range in size from about 15 mμ to 300 mμ in diameter. Viruses are true parasites, in that they have no means of metabolism of their own. They cannot be grown on artificial media, as they need living cells to exist. They are considered by some authorities as falling within the gray area between the lowest forms of life and complex organic chemical molecules.

2-13. MORPHOLOGY AND PHYSIOLOGY OF VIRUSES

a. **Morphology**. Because of its minute size, a virus must be studied with the electron microscope. The virus is much simpler than the bacterial cell, consisting of a core of nucleic acid--either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)--enclosed in a protective membrane of protein (called the capsid). Some viruses have a lipid membrane called an envelope around the outside of the capsid. The shape of viruses varies widely, but in general, they tend to conform to the following:

(1) Viruses of humans, animals, and plants are spherical rod-shaped, or many sided. (Figure 2-7).

(2) Viruses of bacteria (bacteriophages) are shaped like a lunarlander spacecraft. (Figure 2-7).

b. **Physiology**. Being an obligate parasite, the virus requires a living host cell for existence. When a virus comes into contact with a susceptible host cell, it becomes attached to the surface. The virus or its nucleic acid then enters the host cell, where it takes control and utilizes the protoplasm of the host cell to produce new virus particles. The new virus particles then invade additional host cells, and multiplication continues. There is no cell division in the growth and reproduction of viruses. A new virus is formed by chemical synthesis of the viral nucleic acid and capsid proteins (replication). Host cells that have been attacked by a virus may be completely destroyed, or they may suffer little or no harm.

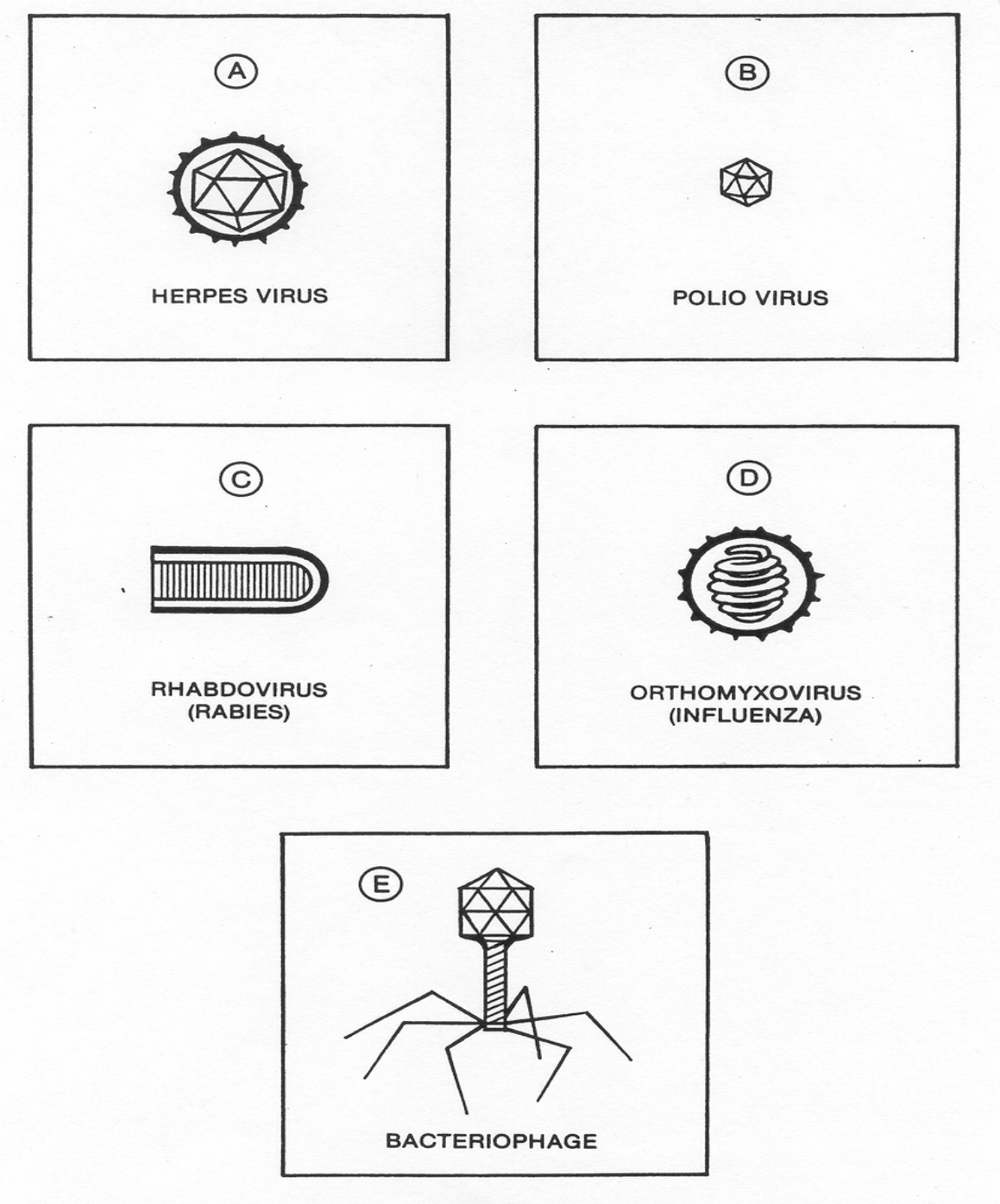


Figure 2-7. Virus forms.

c. **Pathogenesis**. Many virus infections are asymptomatic. On the other hand, some of the most dangerous and highly contagious diseases are caused by viruses. One characteristic of some viruses is found in no ‘other known living disease agent. That characteristic is the ability to cause host cells to proliferate in an abnormal manner, forming growths or tumors (hyperplasia). It is this characteristic which underlies the

current speculation that some cancers may be caused by a virus. Some viruses cause the development of inclusion bodies in the cells they attack. These inclusion bodies may be colonies of the virus, or they may be products formed by the cell in response to the attack. The presence of inclusion bodies is very important in the diagnosis of certain diseases where it is not possible to isolate the virus. In rabies, for example, the presence of Negri bodies (named for their discoverer) in brain cells has been used to confirm the diagnosis, even though the rabies virus may not be isolated.

2-14. BACTERIOPHAGES

Certain viruses have the ability to infect bacterial cells. Such a virus is known as a bacteriophage ("bacteria eater"). Bacteriophages are lunar-lander shaped (figure 2-7(E)) viruses, the action of which is specific for any given species. In other words, coliphages attack only coliform bacteria; staphylophages attack only staphylococci; and so on. Very few bacteria (pneumococci are an example) do not have phages.

a. **Action of Bacteriophages**. The action of bacteriophage on a bacterial. cell is shown in figure 2-8. The virus attaches itself to the bacterial cell (figure 2-8 A), whereupon the tail penetrates the cell wall by chemical action. The DNA of the virus then flows into the bacterial cell (figure 2-8 B). At this point with some viruses the viral DNA joins (links) with the bacterial DNA. Now, whatever genetic code is carried on, the viral DNA will be translated into proteins. Many times these proteins are powerful exotoxins or destructive enzymes. If the viral DNA remains separate from the bacterial DNA then the DNA takes over the action of the cell, synthesizing hundreds of new viruses. When the numerous new phage particles have formed, the cell structure disintegrates, liberating the newly formed virus particles (figure 2-8 C).

b. **Importance of Bacteriophages**. Bacterial viruses occur in nature with their specific hosts. Their highly specific nature assists the bacteriologist in classifying and typing bacteria (an additional identification technique, known as phage typing). Bacteriophages are not always beneficial; in certain industries that depend upon fermentation (baking, wine making, biological, and so forth.), an infestation of bacterial virus could cause great financial loss. Many of the pathogenic effects of bacteria (enzymes and toxins) are produced only when the bacteria are infected with certain viruses.

2-15. PATHOGENIC VIRUSES

Table 2-4 presents a list of the principal viral diseases of public health importance, grouped according to the physiological manifestations, the type of tissue attacked, or the similarity in the infective virus. These groupings are quite arbitrary, in that not all authorities classify these diseases in the same way.

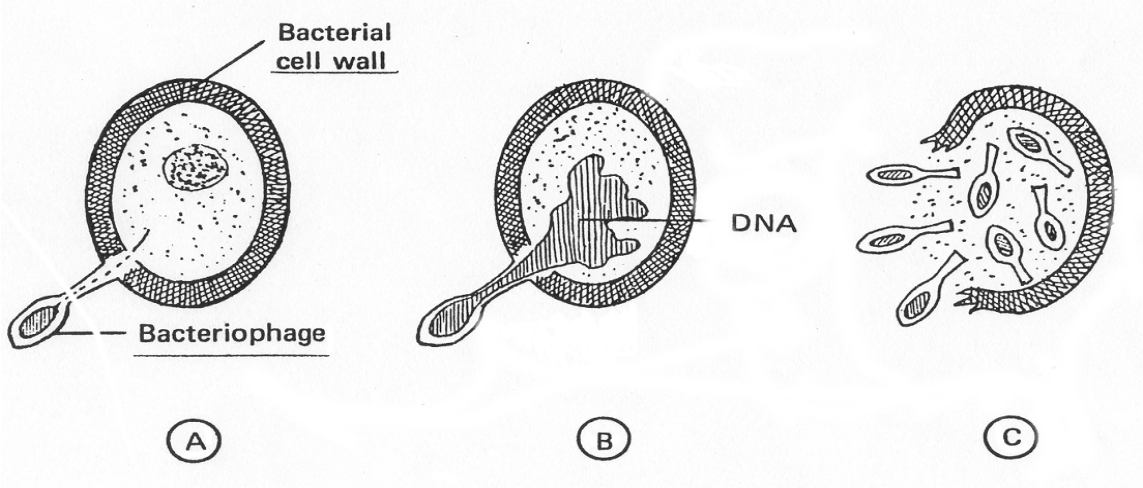


Figure 2-8. Action of bacteriophage on a bacteria cell.

EXANTHEMATOUS (ERRUPTIVE) DISEASES Smallpox (variola) Vaccinia (cowpox) Rubeolla (measles) Rubella (German measles) Herpes simplex (fever blisters, genital lesions) Herpes Zoster (Varicella-Zoster) (shingles) chickenpox Herpes Zoster Molluscum contagiosum (contagious warts) DISEASES OF THE CENTRAL NERVOUS SYSTEM Rhabdovirus (Rabies) (hydrophobia) Poliomyelitis (infantile paralysis) Mumps (epidemic parotitis) [sometimes classified as a respiratory disease] The encephalitides (see arthropod-borne viral diseases) MISCELLANEOUS VIRAL DISEASES Hepatitis A and B Epstein-Barr virus (Infectious mononucleosis) Human T-cell Leukemia Virus (AIDS)	RESPIRATORY DISEASES Rhinovirus and Respiratory Syncytial virus (common cold) Influenza Parainfluenza (croup in children) Viral pneumonias Adenoviruses Acute respiratory infections, eye infections ARTHROPOD-BORNE VIRAL DISEASES Yellow fever Equine encephalitis (a form of sleeping sickness) St. Louis encephalitis Japanese B encephalitis Russian spring-summer encephalitis Sandfly fever Dengue (breakbone fever) Colorado tick fever Epidemic hemorrhagic fever ENTEROVIRUSES ECHO viruses — Cold-like ailments, Coxsackie viruses — certain muscle pains

Table 2-4. Common viral diseases.

Section IV. FUNGI

2-16. GENERAL

Fungi are members of the Plant Kingdom Mycetae (Fungi). Outstanding features of these eucaryptic microorganisms are the presence of cell walls, the lack of motility, and the absence of photosynthesis. Some exist as parasites on other living organisms whereas others are saprophytes, living on the remains of dead plants or animals. The term "fungi" includes the molds, yeasts, mildews, rusts, smuts, mushrooms, and toadstools. This group of organisms includes not only pathogenic species, but also several of important economic value. Molds of the genera Aspergillus and Penicillium are used in the manufacture of antibiotics. Certain molds of the same class are used in the production of cheese (Camembert, Roquefort, and so forth.). Yeasts are used in the production of alcoholic beverages and the baking industry. Edible mushrooms are considered a delicacy by connoisseurs of fine foods.

2-17. MORPHOLOGY OF FUNGI

a. **General**. Fungi vary widely in size and shape, from unicellular, microscopic organisms to multicellular forms easily seen with the naked eye. Individual cells range from 1 µ to 30 µ. Microscopic fungi exist as either molds or yeasts or both. Internally, fungal cells are fairly typical eucaryotic cells.

b. **Molds**. The molds form large multicellular aggregates of long branching filaments, called hyphae. There are vegetative hyphae and reproductive hyphae. Spores are borne on the reproductive hyphae. (Fungal spores should not be confused with bacterial spores that are resistant bodies formed for bacterial survival rather than reproductive purposes.) Spore size, shape and structure are used in the classification and identification of fungi. The tube-like hyphae are responsible for the fluffy appearance of the macroscopic mold colony. The hyphae and other structures combine to form an elaborate network called a mycelium.

c. **Yeasts**. These are large (5 to 8 µ), single-celled organisms that rarely form filaments. Most yeasts reproduce by the asexual process of budding. Yeast colonies are usually characterized by a smooth surface similar to that of many bacteria.

2-18. PHYSIOLOGY OF FUNGI

a. **Nutrition**. Most fungi contain complex enzymes and other chemical substances which, when diffused into the host, break down the complex substances available--wood, vegetation, leather, bread, and so forth--into simpler substances that can be used for food. The chemical products of digestion are, therefore, completed outside of the organism, and the fungus absorbs the end products.

b. **Reproduction**. Fungi reproduce sexually or asexually, or both, depending upon the species and the environmental conditions. As the name implies, sexual

reproduction is the result of the union of two spores. Most fungi reproduce both sexually and asexually. Those that produce only asexual spores are known as Deuteromycetes Fungi imperfecti. This group is important because it contains most of the pathogenic fungi. The yeasts reproduce both by spores and by a process known as budding, which is similar to binary fission. The yeast cell forms a small knoblike protrusion, or bud (figure 2-9), that separates from the mother cell and grows until it reaches full size, at which time the process is repeated.

c. **Growth**. Fungi grow well under the same conditions that favor the growth of bacteria--warmth and moisture. It is for this reason that fungal infections pose a serious problem to troops in the tropics. As the temperature decreases, fungal activity also decreases; however, the spores are very resistant to cold, some surviving freezing temperatures for long periods of time. On the other hand, fungi are easily killed at high temperatures.

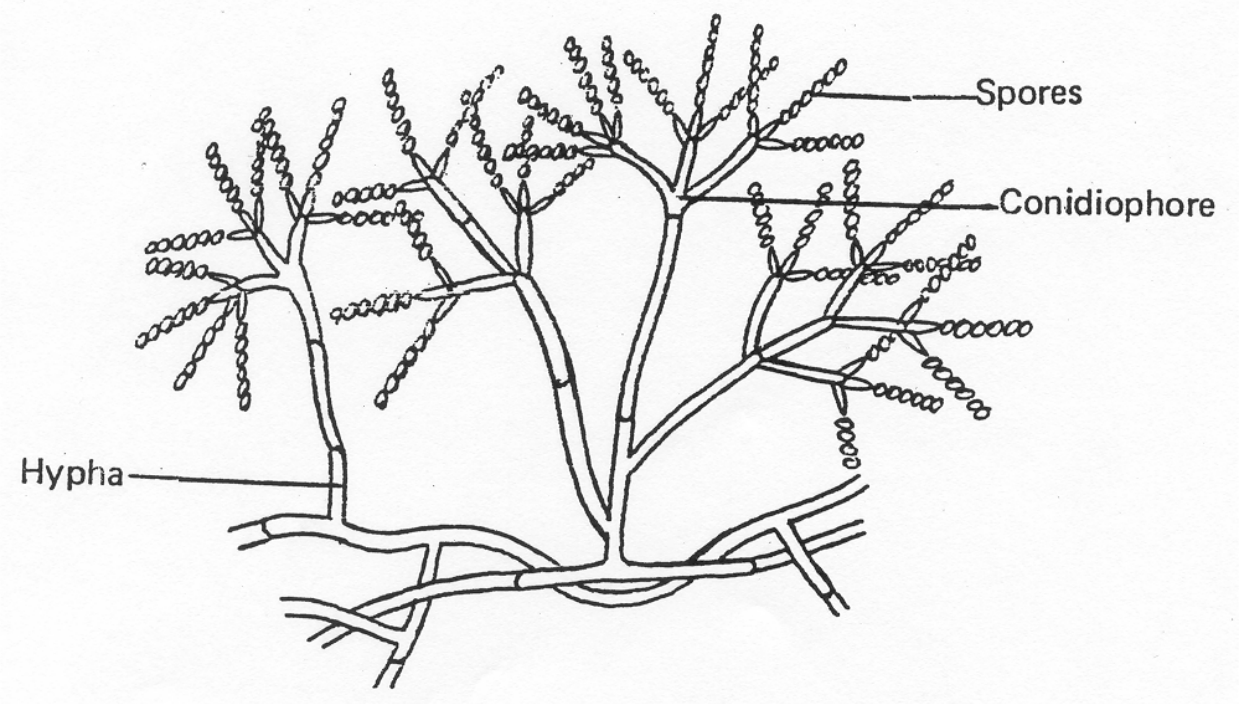


Figure 2-9. Typical mycelium of a fungus.

2-19. CLASSIFICATION OF FUNGI

Fungi are usually classified according to biological taxonomy based upon the type of hypha, spore, and reproduction. There are four classes of fungi, whose characteristics are shown in Table 2-5 and figure 2-10.

a. **Class Phycomycetes**. The algal fungi: bread molds and leaf molds. The only known mycosis (fungal disease) caused by fungi of this class is mucormycosIs, a very rare fungal growth of the upper respiratory tract, bronchial mucosa, and lungs. It occurs largely as a complication of a chronic, debilitating disease, such as uncontrolled diabetes.

b. **Class Ascomycetes**. The sac fungi: yeasts, mildews, and cheese molds. Fungi of this class are implicated in only three fungus diseases, all of which are rare.

c. **Class Basidiomycetes**. Mushrooms, toadstools, rusts, and smuts. The only pathogens in this class are the mushrooms of the genus Amanita, which cause severe systemic poisoning (sometimes death) when eaten.

d. **Class Deuteromyceters**. Fungi imperfecti: a heterogeneous collection of fungi without sexual reproduction. Most of the pathogens encountered in medical mycology belong to this class.

Taxonomic class of Fungi	**Hypha**	**Type of Repro-duction**	**Character-istic spore**	**Origin of Spore**	**Examples of Fungi**	**Patho-genicity**
Phycomycetes	Asptate	Asexually	Sporangio-spore	Sporangio phore	Nuisance fungi including general	Very rare Mucormycosis
		Sexually	Zygospore or oospore	Fussion of nuclei	Absidia, Muclor, and Rhizopus	
Ascomycetes	Septate	Asexually	Blastospore	Budding	Allescheria Aspergillus	Rare Maduromcosis
			Conidium	Conidio-phore	Piedraia	Aspergillosis Black Piedra
		Sexually	Ascospore	Ascus	Saccharomyces (perfect yeast)	
Basidiomycetes	Septate	Sexually	Basidio-spore	Basidium	Mushrooms, smuts and rusts	Rare Mushroom poisoning
Deutero-mycetes {fungi imperfecti)	Septate	Asexually	Thallospore	Thallus (hypha)	Most saprophytes and pathogens encountered in medical mycology (Imperfect mold and yeast)	Most Mycoses encountered in medical mycology
			Conidium	Conidio-phore		

Table 2-5. Characteristics of Fungi.

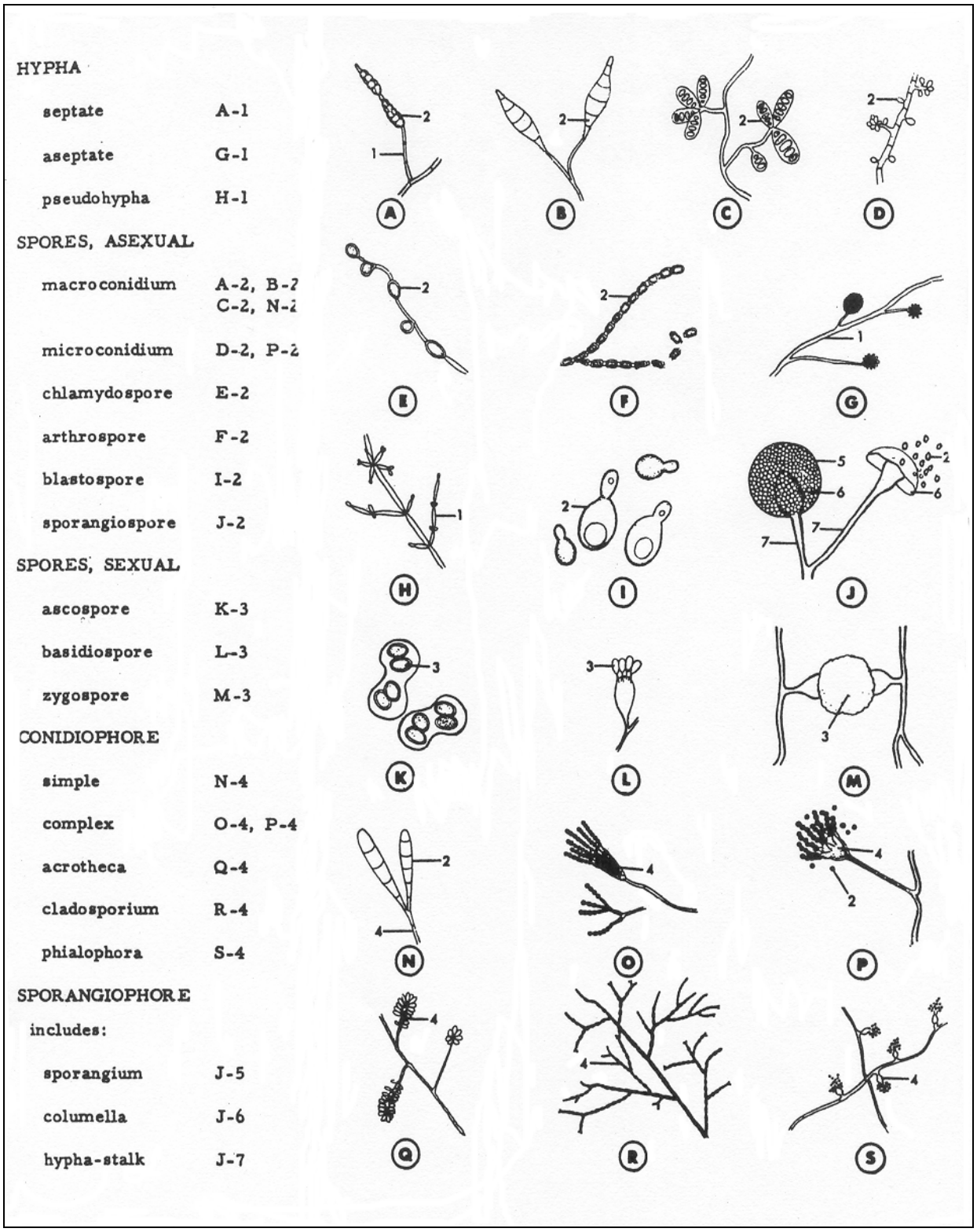


Figure 2-10. Structural components of fungi.

2-20. PATHOGENIC FUNGI

a. Fungal infections are of two types: localized skin infections (dermatomycoses), and systemic infections. Although the former are far more common, the latter generally have more serious consequences. Table 2-6 lists the more common fungus diseases and the important etiological agents in each. Note that frequently more than one species of organism may cause identical symptoms.

Disease	**Synonym or Brief Description**	**Important Etiological Agents**
	CUTANEOUS AND SUPERFICIAL MYCOSES	
Tinea capitis'	Ringworm of the scalp	*Microsporum* spp *Trichophyton* spp
Tinea corporis	Ringworm of the body	Same as *Tinea capitis*
Tinia barbae	Infection of bearded area of face and neck	*Trichophyton* spp
Tinea cruris	Ringworm of the groin (jock itch)	*Trichophyton* spp *Candida albicans* *Epidermophyton floccosum*
Tinea pedis	Ringworm of the feet (athlete's foot)	Same as *Tinea cruris*
Tinea versicolor	Depigmented, scaly patches of skin	*Malassezia furfur*
Otomycosis (aspergillosis)	Fungus infection of the ear canal	*Aspergillus* spp
Cutaneous Candidiasis (moniliasis, thrush)	Yeast infection of nails, skin, mouth, Vagina	*Candida albicans* and other species
Mycetoma	Tumor-like swelling, draining abscess	*Pseudallescheria boydii* and other
Actinomycosis	Chronic, suppurative or granu-lomatous disease of jaw, thorax, or abdomen	*Actinomyces israelii* [actuallyclassified as bacteria, but cause fungus-like infections]

Table 2-6. Common fungus diseases. (continued)

Disease	Synonym or Brief Description	Important Etiological Agents
SUBCUTANEOUS AND SYSTEMIC FUNGUS INFECTIONS		
Nocardiosis	Infection of lungs, other organs, and lower extremities (Madura foot)	*Nocardla asteroids* [actuallyclassified as bacteria, but cause fungus-like infections] *N. brasIIIensIs*
Chromoblastomycosis	Warty nodules or vegetations of skin and subcutaneous tissues	*Cladosporium carrionii* *Fonsecaea pedrosoi*
Sporotrichosis	Ulcers of skin and underlying tissues and gumma-like swelling of regional lymph nodes.	*Phialophora verrucosa* *Sporot schenkii*
Blastomycosis	Inflamatory lesions of the skin, lungs, or bones.	*Blastomyces dermatltidis*
Coccidioidomycosis	Self-limited respiratory disease or chronic progresssive infection of various organs	*Coccidioides immitis*
Histoplasmosis	Fungus infection of the lungs, with fever; anemia; loss of weight, enlargement of lymph nodes, liver, spleen	*Histoplasma capsulatum*
Cryptococcosis	Systemic fungus infection of lungs or meninges	*Cryptococcus noeformans*

Table 2-6. Common fungus diseases (concluded)

b. Diseases caused by fungi are collectively called mycoses (singular, mycosis). They are divided into four general categories on the basis of the primary tissue affected by the pathogen:

(1) Superficial mycoses are infections limited to the hair and dead layers of the skin.

(2) Cutaneous mycoses (dermatophytoses or ringworm) affect only the skin, hair, and nails.

(3) Subcutaneous mycoses affect the subcutaneous tissue below the skin and occasionally bone.

(4) Systemic ("deep") mycoses infect the internal organs and may spread throughout the host

c. Those fungi infecting the outer layers of the skin are rarely severe and are usually transmitted by contact with infected animals or humans. The agents of subcutaneous and systemic mycoses, however, are normally saprophytic fungi growing in the soil. Humans generally acquire these mycoses only when the spores of these organisms are either inhaled or introduced into the body through a break in the skin.

d. Some fungi incapable of causing infectious diseases produce toxic substances that poison the person who ingests them. These substances are collectively called mycotoxins. The most commonly known mycotoxin poisoning is from certain mushrooms; however, mycotoxins may be produced by fungi growing on grain, nuts, and other agricultural products.

Section V. PROTOZOA

2-21. GENERAL

Protozoa are minute, unicellular organisms belonging to the Animal Kingdom Protista. They range in size from nearly submicroscopic 100u; however, the vast majority of them are microscopic. Thousands of species have been identified, but relatively few are pathogenic to man.

a. **Morphology**. Protozoa vary widely in structure. Some have organelles of locomotion--flagella, cilia, or pseudopods. Some have definite openings to absorb food. All consist of one or more nuclei and cytoplasm. Many have various inclusion bodies.

b. **Physiology**.

(1) Nutrition. Most protozoa absorb fluid directly through the cell membrane. Many can take in solid food and digest it by enzymes produced by their own cytoplasm.

(2) Reproduction. Protozoa multiply species, and by sexual reproduction in others. of sexual and asexual reproduction.

(3) Locomotion. Nearly all protozoa possess some means of locomotion. Some have flagella (figure 2-6); others have cilia (delicate, hair-like organelles); and some have pseudopods (false feet), which are protoplasmic processes that flow forward in a manner similar to the action of a balloon partially filled with liquid, pulling the cell along with them.

(4) Cyst formation. Many protozoa have the ability to form cysts when subjected to an unfavorable environment. This process is similar to spore formation in bacteria. Since protozoan cysts are much more resistant to unfavorable

environmental conditions than are the vegetative forms, the cysts are responsible for the transmission of most protozoan infections.

2-22. CLASSIFICATION OF PROTOZOA

The pathogenic protozoa of man can be grouped into four major classes:

a. **Class Rhizopoda (ameba)**. These organisms are characterized by finger-like protoplasmic processes known as pseudopods (false feet), which serve as organs of locomotion and for the engulfment of food. They multiply by binary fission during the motile stage, in which they are known as trophozoites. Many species form cysts, which consist of several nuclei and a resistant membrane (figure 2-11B). The trophozoites are very susceptible to injurious agents and are easily destroyed; however, the cysts are quite resistant. The cysts, therefore, are responsible for transmitting disease. Amebae of man are found primarily in the digestive tract.

b. **Class Ciliata (ciliates)**. The ciliates are characterized by an oval, uniformly shaped cell having two nuclei (macronucleus and micronucleus) and numerous short, hair-like appendages (cilia) for locomotion (figure 2-12 A). Some species multiply by binary fission and some by conjugation. Cysts are not commonly encountered, but when found, they are double walled, spherical bodies. The only species pathogenic to man is *Balantidium coli*, a natural inhabitant of the large intestine of the domestic hog.

c. **Class Mastigophora (flagellates)**. The flagellates are distinguished by having, at some time during the life cycle, relatively long, filamentous protoplasmic processes of locomotion known as flagella (figure 2-13 A). The flagella may be multiple or single. Multiplication is by binary fission. Some species produce cysts, while others do not. Flagellates of man are normally found in the intestinal tract, the genital tract, and the circulatory system.

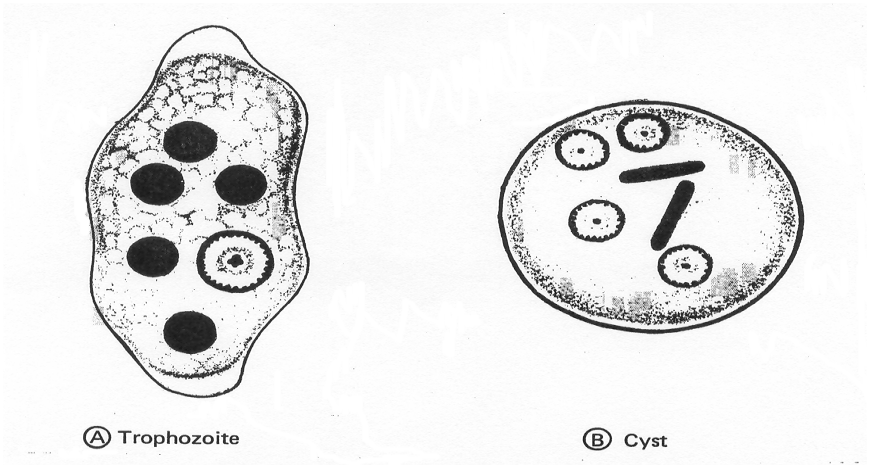


Figure 2-11. *Entamoeba histolytica.*

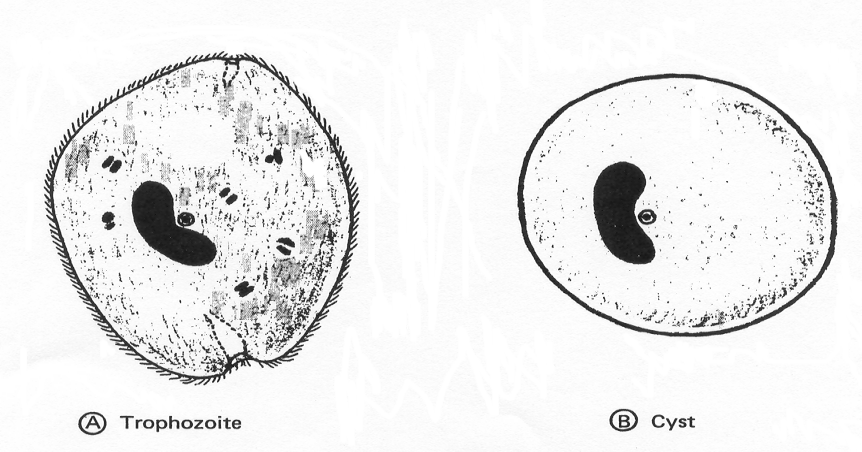


Figure 2-12. *Balantidium coli.*

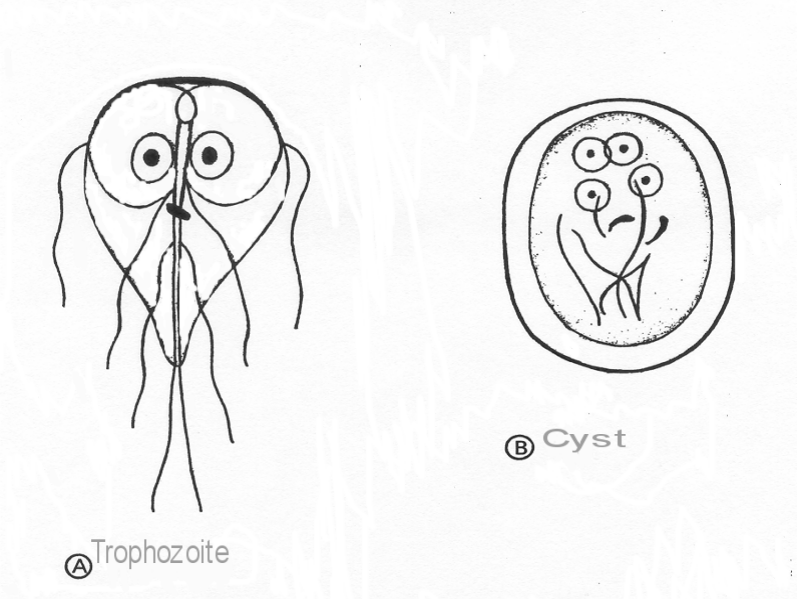


Figure 2-13. *Giardia lamblia.*

2-23. PATHOGENIC PROTOZOA

Table 2-7 lists the principal protozoan diseases according to the classification of the infectious agent. Figure 1-14 shows the life cycle of the malarial parasite.

INFECTIOUS AGENT	DISEASE OR CONDITION
AMEBAE	
Entamoeba histolytica	Amebic dysentery (amebiasis)
CILIATES	
Balantidium coli	Bloody dysentery similar to amebiasis
FLAGELLATES	
Giardia lamblia	Diarrhea, malabsorption
Trichomonas vaginalis	Inflammatory changes in the vagina (vaginitis)
Trypanosoma brucei	
Trypanosoma Modesiemse gambiense	African sleeping sickness.
Trypanosoma. cruzi	Chagas' disease (American trypanosomiasis), a systemic infection resulting in spleen and liver enlargement and myocardial damage.
Leishmania donovani	Kala-azar (tropical splenomegaly), a disease characterized by fever, disturbances in the blood-forming organs, and enlargement of spleen and liver.
Leishmania tropica	Cutaneous leishmaniasis (tropical sore)
Leishmania braziliensis	American leishmaniasis (mucocutaneous' leishmaniasis or espundia), ulcerative necrosis of nose, mouth, and pharynx.
SPOROZOA	
Plasmodium vivax	Vivax (benign tertian) malaria.
Plasmodium malariae	Quartan malaria.
Plasmodium £alciparum	Falciparum (aestivo-autumnal or malignant tertian) malaria.
Plasmodium ovale	Ovale malaria (rare)

Table 2-7. Principal protozoan diseases of man.

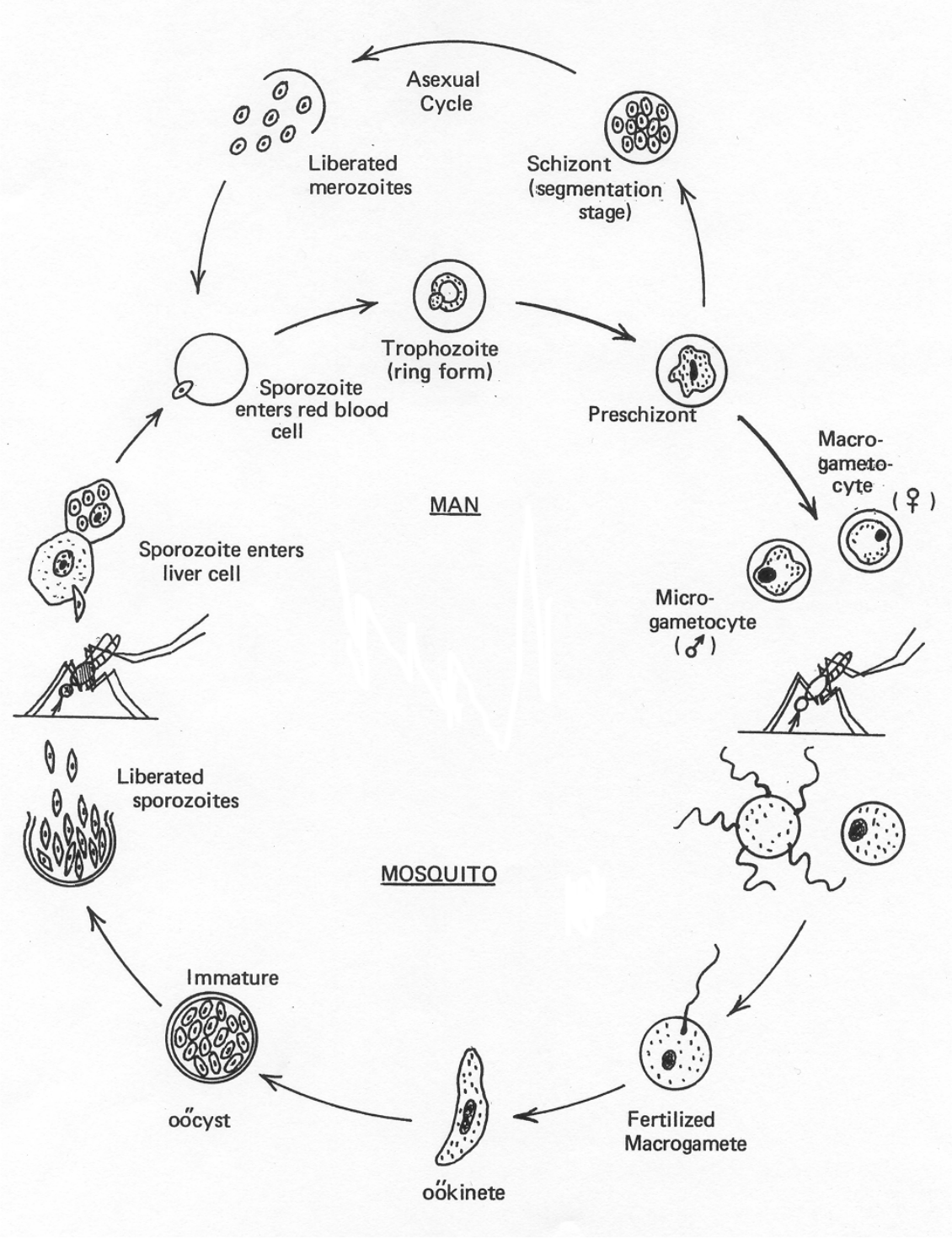


Figure 2-14. Life cycle of the malarial parasite.

2-24. GENERAL

Helminths are multicellular (Metazoa) worms or wormlike animals. They may be parasitic or free living. Since they are multicellular, most helminths may be easily seen with the naked eye in the adult form and are not truly within the scope of microbiology. Because of their medical importance, however, helminths are usually studied, along with protozoa, as part of the science of medical parasitology.

2-25. CLASSIFICATION OF HELMINTHS

Helminths are found in two phyla of the subkingdom Metazoa (multicellular animals):

a. Phylum: Platyhelm1nthes (flatworms)

(1) Class I: Cestoda (tapeworms)

(2) Class II: Trematoda (flukes)

b. Phylum: Aschelminthes

Class I: Nematoda (roundworms, threadworms)

2-26. CESTODES (TAPEWORMS)

a. The tapeworm has two stages: larva and adult. The length of an adult worm varies, according to the species, from 3-8 mm to 25 or 30 feet. The adult form has a small head (scolex) and varying numbers of segments (proglottides) (figure 2-15 A&B). The head attaches itself to the intestinal wall of the host by means of suckers. Segments form from the head, receiving their nourishment through absorption, since the worm has no alimentary tract. Each mature segment is a sexually complete hermaphrodite (possessing both male and female sex organs), capable of producing thousands of eggs. Treatment that fails to dislodge the head is ineffective, since the head will immediately begin to replace the lost segments. In most instances a cestode requires both an intermediate host (where certain stages of development occur) and a definitive host (where the adult worm lives and produces ova) to complete its life cycle. Table 2-8 lists the principal cestodes affecting man.

b. When eggs or segments of most tapeworms are passed from the definitive host (man), they are ingested by the intermediate hosts (ruminants, swine, and so forth) while grazing or rummaging for food. The larval stage, a hexacamth oncosphere, hatches and encysts as a cysticercus or cysticercoid larva in the muscles or various organs of the intermediate host. Therefore, the eggs are infective to man and may encyst in tissue as a cysticercus larva resulting in severe complications. In the life cycle of the large fish tapeworm of humans, the eggs must reach water where a larval stage (coracidium) emerges and is swallowed by a small fresh-water crustacean called a

copepod that is the first intermediate host. Finally, the infective larval stage (a plerocercoid) developes and encysts in the tissues of the fish. In the life cycle of most tapeworms the infective larval stages attach to the small intestine and become adults when man consumes the improperly prepared flesh of the various intermediate hosts.

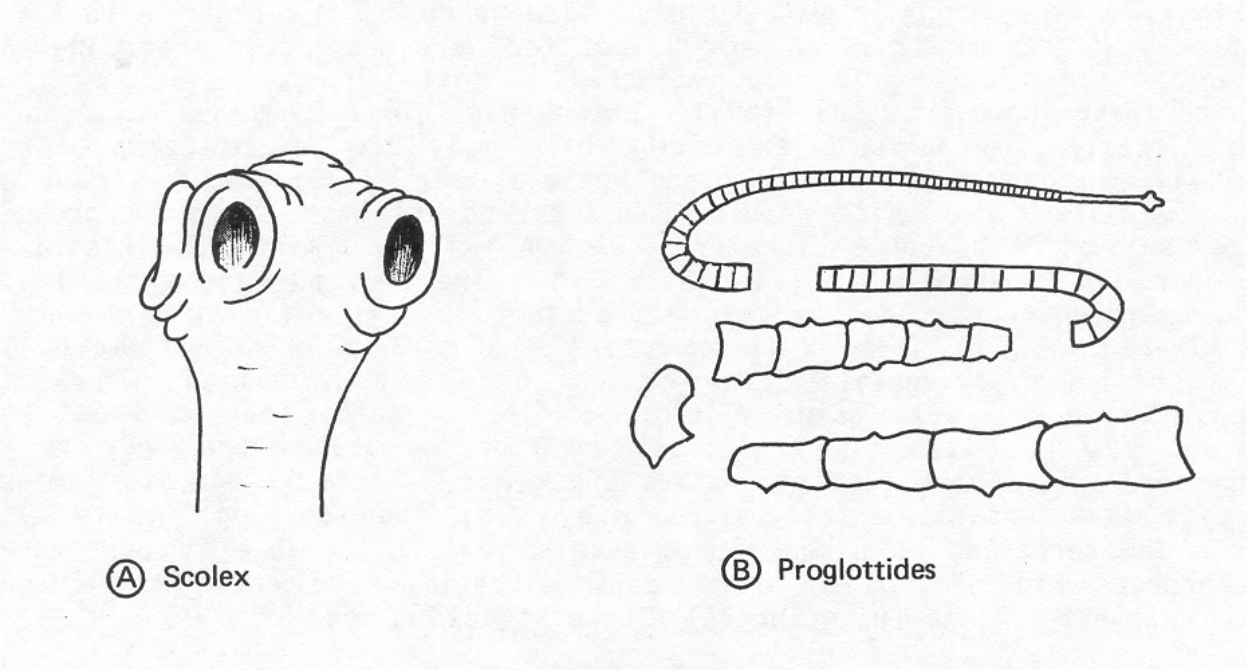


Figure 2-15. Tapeworm.

CESTODE	COMMON NAME	HOST INTERMEDIATE	HOST DEFINITIVE
Taeniarhynchus saginatus	Beef tapeworm	Cattle	Man
Taenia solium	Pork tapeworm	Pig	Man
Echinococcus granulosus	Dog tapeworm	Man, Sheep hog, cattle	Dog,
Diphyllobothrium latum	Fish tapeworm	Crustacean, fish	Man, cat, bear, dog
Hymenolepis nana	Dwarf tapeworm	Not necessary	Man

Table 2-8. Principal cestodes affecting man.

2-27. TREMATODES (FLUKES)

Flukes are small, nonsegmented, leaf-shaped parasites equipped with "suckers" which enable them to attach themselves to the lumina of the intestines, liver, lungs, and other organs. They are referred to as intestinal, liver, lung, or blood flukes, depending on the organ affected. Some flukes attack more than one organ, but they are designated according to the organ that is primarily attacked. Most flukes are complete hermaphrodites; however, one family pathogenic to man--Schistosomatidae (blood flukes)--is dioecious, exhibiting both male and female forms. The adults live in copula within the blood vessels (figure 2-16). Flukes have a complicated life cycle that requires an intermediate host (certain species of water snails). The adult fluke (figure 2-16 (A)) lives within the definitive host, where it produces eggs (figure 2-16 (B)). The eggs pass from the host (usually through feces), and if water is present, a minute larval form known as a miracidium (figure 2-16 (C)) emerges. The miracidium swims until it finds a snail (or perishes). It penetrates the intermediate host, where it develops into a cercaria (figure 2-16 (D)). The cercaria emerges from the snail and attaches itself to aquatic plants or penetrates the body of a second intermediate host (certain fishes and crabs), where it encysts. When the cysts (metacexcariae) are swallowed by man, they develop into adult flukes. The cercariae of blood flukes (schistosomes) are able to penetrate the unbroken skin of man or other definitive host and enter the blood stream. Table 2-9 lists the principal flukes affecting man.

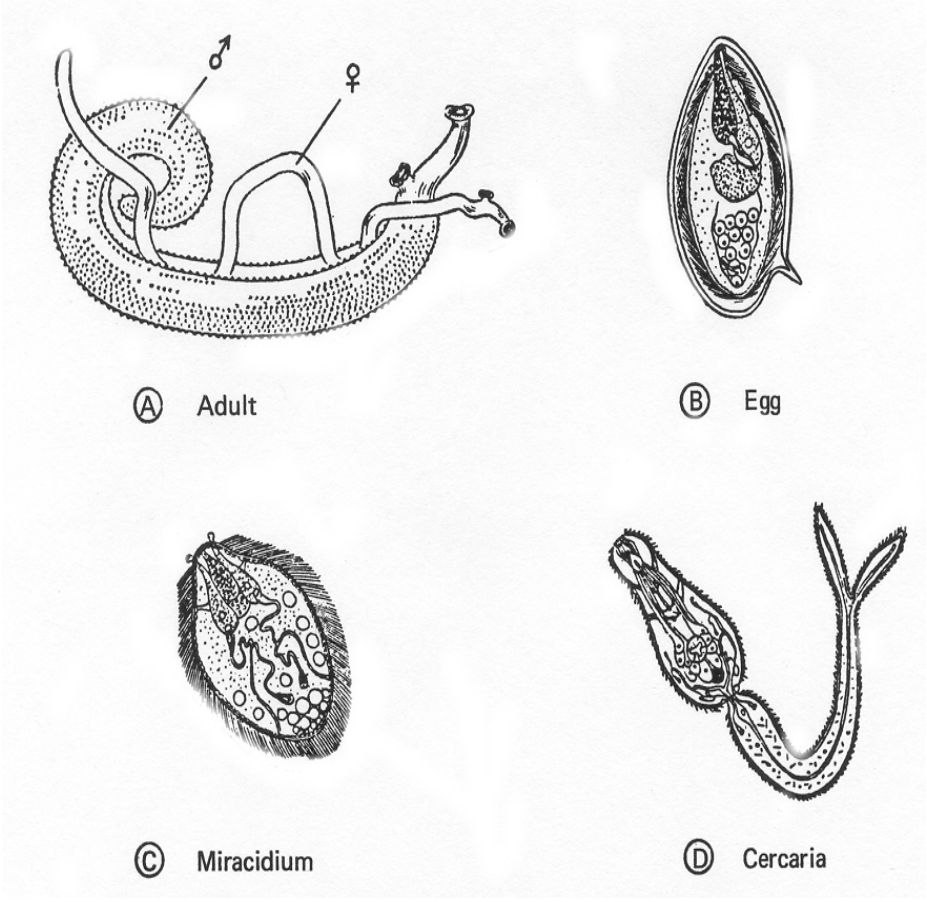


Figure 2-16. Fluke (Schistosoma mansoni)

TREMATODE INFESTATION	INTERMEDIATE HOST(S}	MODE OF ENTRY	SITE OF
Schistosoma hematobium		Penetration of skin by free-swimming cercariae.	Blood vessels of urinary bladder
Schistosoma mansoni	Snail		Inferior mesenteric veins
Schistosoma japonicum			Superior mesenteric veins
ParaQonimus westermani	Snail-crab	Ingestion of infested crabmeat	Lungs
Opisthorchis sinensis	Snail-fish	Ingestion of infested fish	Liver (bile ducts)
MetaQonimus yokegawai	Snail-fish	Ingestion of infested fish	Intestine
Heterophves heterophyes	Snail-fish	Ingestion of infested fish	Intestine
Fasciola hepatica*	Snail	Ingestion of encysts larvae on vegetation	Liver (bile ducts)
Fasciolopsis buski**	Snail	Ingestion of encysts larvae on vegetation	Intestine
Gastrodiscoides hominis**	Snail	Ingestion of encysts larvae on vegetation	Intestine

*Usual definitive host is sheep, but man is sometimes affected.

**Usual definitive host is swine, but man is sometimes affected.

Table 2-9. Principal trematodes affecting man.

NOTE: Table 2-10 below refers to 2-28. Nematodes (Roundworms) found beneath Table 2-10.

NEMATODE	HOST(S)	MODE OF ENTRY	USUAL SITE OF INFESTATION
Anyclostoma duodenale) (Old World hookworm) Necator americanus (New World hookworm)	Man	Through the skin of a barefoot person	Small intestine
Ascaris lumbricoides (large roundworm)	Man	Food or drink contaminated with feces by flies or fingers	Small intestine (also lungs, trachea, and other organs
Enterobius (Oxyuris) *vermdcularis* (pinworm)	Man	Ingestion through contaminated hands, clothing, bed linen, etc.	Intestine (females migrate to anus to deposit eggs)
Strongyloides stercoralis	Man	Active penetration of larvae through the skin	Intestinal mucosa
Trichuris trichiura (whipworm)	Man	Contaminated food or drink	Cecum, appendix, upper colon
Trichinella spiralis	hog, Rat, man	larvae imbedded in Ingesting encysts flesh	encystment in Intestinal tract muscle fibers
Wuchereria bancrofti (filarial worm) *Brugia malayi* (filarial worm)	Man	Through the bite of a mosquito harboring the larvae	Lymphatic system and connective tissue
Onchocerca volvulus (blinding filarial worm	Man	Through the bite of the black fly or buffalo gnat	Skin and sub-cutaneous tissue, especially of head and neck
Loa. loa (African eye worm)	Man	Through the bite of a fly	Subconjunctival and subcutaneous tissue
Dracunculus medinensis (guinea worm)	Man	Ingestion of infected water fleas (copepods)	Skin of the legs

Table 2-10. Principal nematodes affecting man.

2-28. NEMATODES (ROUNDWORMS)

Nematodes are cylindrical worms with tapered bodies, resembling earthworms in appearance. They range in length from about 2 mm, in the case of Strongyloides stercoralis, to about 40 cm., in the case of Ascaris lumbricoides (the intestinal roundworm). Females of Dracunculus medinensis may even attain the length of 120 cm. The sexes are usually distinct, the females being larger than the males. With the exception of the filariae, these parasites do not require an intermediate host. They are transmitted from one host to another primarily by contamination of fingers, food, fomites, and soil with infected feces. The life cycle consists of three stages: eggs or embryos, larva stages, and adult worms. The eggs of most nematodes are passed from the definitive host in feces and, as they mature, rhabditoid larvae develop which soon become infective stage larvae. Mature eggs, when ingested by a suitable definitive host, disintegrate releasing the infective larvae which find their habitat in the new host systems; some of them maturing in the lungs before finally seeking their habitat in the intestine. A notable exception occurs in the life cycle of the hookworms where the infective stage larvae are capable of entering the host by active penetration of the skin. Then they migrate via the circulatory system to the lungs and ultimately find their habitat in the small intestine. Another exception, the filarial worms instead of producing eggs, produce larvae that swarm into the blood where mosquitoes while taking a blood meal from the host, ingest these larvae and later transmit them to a new host. Larvae produced by the guinea worms rupture from blisters formed on the skin of the host and escape into water where they are ingested by fresh-water crustaceans (copepods) that are in turn swallowed by new definitive hosts. The effect of the various hematodes on man may vary from mild, unnoticed symptoms to severe illness and death. Table 2-10 (above) lists the principal nematodes affecting man and the usual site of infestation.

Continue with Exercises

EXERCISES, LESSON 2

INSTRUCTIONS. The following exercises are to be answered by marking the lettered response that best answers the question or by completing the incomplete statement or by writing the answer in the space provided.

After you have completed all the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers.

1. Microscopic forms of life are known as _____.

2. Microorganisms that cause disease in man or animal are known as _____.

3. The basic unit, or building block, of all living matter is the _____.

4. The simplest form of cell division is _____.

5. The action of a living cell and its hereditary characteristics are governed by the _____.

6. The metabolic processes of a living cell take primarily in the _____.

7. Bacteria are:

   a. Oblong parasites.

   b. Unicellular organisms of the Kingdom Animalia.

   c. Unicellular organisms of the Kingdom Monera.

   d. The smallest known form of life.

8. Bacteria reproduce by:

    a. Mitosis.

    b. Meiosis.

    c. Binary fission.

    d. Sexual intercourse.

9. Bacteria that appear as chains of spherical objects are:

    a. Bacilli

    b. Streptococci

    c. Gonococci.

    d. Staphylococci.

10. Which of the following are means of locomotion?

    a. Flagella.

    b. Spores.

    c. Capsules.

    d. Inclusion bodies.

11. Which of the following is the least reliable means of identifying bacteria?

    a. Appearance.

    b. Food and oxygen requirements.

    c. Staining characteristics and food requirements.

    d. Productivity.

12. Microorganisms that grow on dead organic matter are known as _____.

13. Which of the following diseases is caused by a gram-positive, aerobic, acid-fast bacillus?

    a. Typhoid fever.

    b. Tetanus.

    c. Tuberculosis.

    d. Diphtheria.

14. Which of the following diseases is caused by a spirochete?

    a. Syphilis.

    b. Gonorrhea.

    c. Leprosy.

    d. Cholera.

15. Viruses are:

    a. Saprophytes.

    b. Facultative aerobes.

    c. Obligate intracellular parasites.

    d. Obligate anaerobes.

16. Viruses have no means of metabolism on their own; therefore, they are considered to be true_____.

17. Viruses multiply by:

    a. Binary fission.

    b. Replication in the host cell

    c. Mitosis

    d. Sexual reproduction

18. Viruses that attach bacterial cells are known as _____.

19. Which of the following diseases are caused by viruses? (Mark more than one.)

    a. Rabies

    b. Typhoid fever.

    c. Hepatitis

    d. Malaria.

    e. Yellow fever.

    f. Polimyelitis.

    g. Shigellosis.

20. Which of the following characteristics apply to rickettsiae?

    a. Smaller than viruses.

    b. Obligate parasites.

    c. Multiply by binary fission.

    d. Smaller than typical bacteria.

    e. Normally transmitted through arthropods.

    f. Normally pathogenic to arthropod hosts.

    g. Uniform in shape within species.

21. Fungi are members of the _____ Kingdom.

22. The intricate vegetative network of a fungus or yeast is known as a _____.

23. Which of the following diseases are caused by fungi?

    a. Leprosy.

    b. Aspergillosis.

    c. Ringworm.

    d. Actinomycosis,

    e. Gonorrhea.

24. Protozoa are members of the _____ kingdom.

25. Which of the following characteristics apply to protozoa?

    a. Usually possess means of locomotion.

    b. Normally visible to the naked eye

    c. Multicellular.

    d. Form cysts for protection.

    e. Generally larger than bacteria.

26. Amebae of man is found primarily in the _____.

27. The only ciliate pathogenic to man is _____, a natural inhabitant of the of the _____ of the _____.

28. Flagellates of man are usually found in the:

    a. _____ tract.

    b. _____ tract.

    c. _____ system.

29. The three classes of helminths of public health importance are:

    a. _____.

    b. _____.

    c. _____.

30. Cestodes normally have two stages: _____ and _____.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 2

1. Microorganisms. (para 2-1a)
2. Pathogenic. (para 2-1c)
3. Cell. (para 2-2)
4. Binary fission. (para 2-3e)
5. Nucleus. (para 2-2b(2))
6. Cytoplasm. (para 2-2b(1»
7. c (para 2-6)
8. c (para 2-3e)
9. b (para 2-7a(3»
10. a (para 2-8b(2»
11. a (para 2-9)
12. Saprophytes. (para 2-9a(1»
13. c (Table 2-2)
14. a (Table 2-2)
15. c (para 2-12)
16. Parasites. (para 2-12)
17. b (para 2-13b)
18. Bacteriophages. (para 2-14)
19. a, c, e, f. (Table 2-3)
20. b, c, d, e. (para 2-11)
21. Plant. (para 2-16)
22. Mycelium. (Figure 2-9)

23. b, c, d. (Table 2-6)

24. Animal. (para 2-21

25. a, d, e. (para 2-21b)

26. Digestive. (para 2-22a)

27. Balantidium coli,
Large intestine,
swine (hog) (para 2-22b)

28. a. Intestinal.
b. Genital.
c. Circulatory. (para 2-22c)

29. a. Cestodes.
b. Trematodes.
c. Nematodes. (para 2-25)

30. Larval; adult. (para 2-26)

End of Lesson 2

LESSON ASSIGNMENT

LESSON 3	Practical Application of Microbiology.
TEXT ASSIGNMENT	Paragraphs 3-1 through 3-7.
LESSON OBJECTIVE	Upon completion of this lesson, you should be able to:

3-1. Identify those microorganisms that are important in water and sewage treatment.

3-2. Identify methods and materials used for disinfection and sterilization.

3-3. Identify proper procedures for packaging microbiological specimens for shipment.

SUGGESTION After completing the assignment, complete the exercises of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 3

PRACTICAL APPLICATION OF MICROBIOLOGY

Section I. MICROBIOLOGY OF WATER AND SEWAGE

3-1. INTRODUCTION

a. **General**. Water is probably man's most precious commodity. Without water, no living organism can exist. Man's health and comfort are dependent upon a safe and palatable water supply. Pure water is seldom found in the natural state. Virtually all water that is available in sufficient quantity for man's needs contains impurities. Most of these impurities are harmless, although they may affect the appearance and palatability of the water. Natural occurring water may contain any of the following impurities:

(1) Inorganic chemicals--primarily metals, metallic salts, and gases.

(2) Suspended solids:

(a) Silt and nonliving organic matter.

(b) Plant life.

(c) Animal life.

b. **Plant Life**. In addition to the microorganisms previously discussed in this chapter--viruses, bacteria, and fungi--the plant forms known as algae are of interest to the sanitary microbiologist. Algae are those microscopic plants--both unicellular and multicellular--which have chlorophyll but no stems or leaves. They are important in water microbiology because they impart tastes, odors, colors, and turbidity to water.

c. **Animal Life**. The animal microorganisms of concern to sanitary microbiologist are protozoa, rotifers, crustaceans, and helminthes.

(1) Rotifers. Rotifers are among the simplest of multicellular animals. The rotifer gets its name from two sets of cilia at one end that gives the appearance of two contra rotating wheels (figure 3-1). The cilia are dual purpose--for mobility and food catching. The rotifer's chief source of food is bacteria. It is a strict aerobe and is found only in water that contains low organic content and sufficient dissolved oxygen for respiration. This is a good indication that the water is low in pollution.

(2) Crustaceans. Most crustaceans, such as crayfish and lobsters, are macroscopic (visible with the naked eye); however, some are microscopic. Two crustaceans of interest to the sanitary microbiologist are Daphnia and Cyclops (figure 3-2). They feed on algae and bacteria and are an important source of food for

fish. Because they are strict aerobes, they are also indicators of relatively stable streams or lakes. Crustaceans have been used to reduce the level of algae in bodied or water.

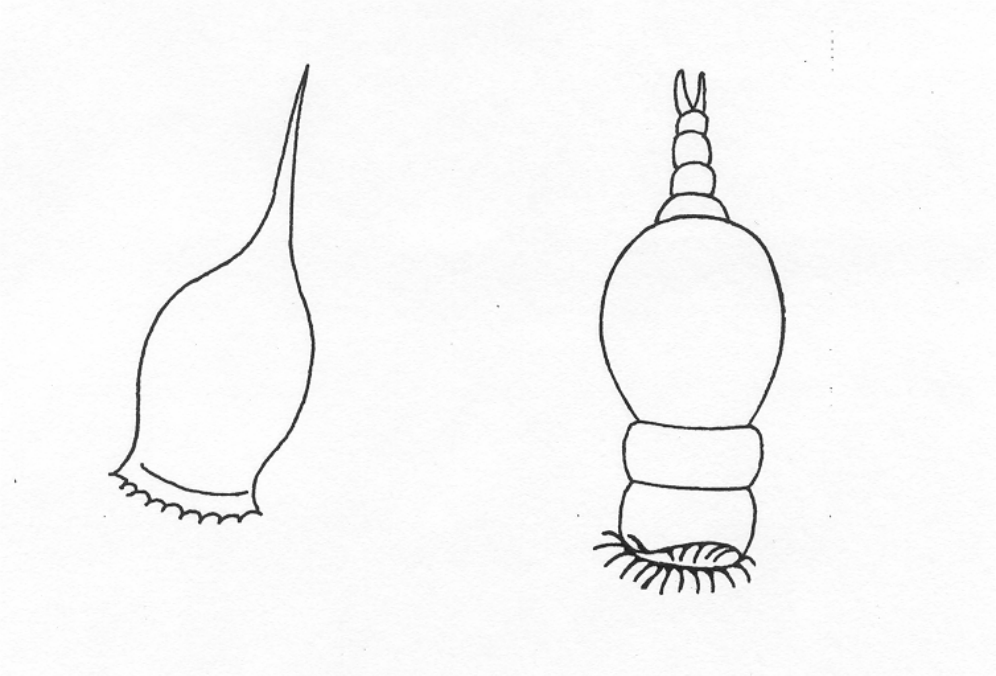


Figure 3-1. Rotifera.

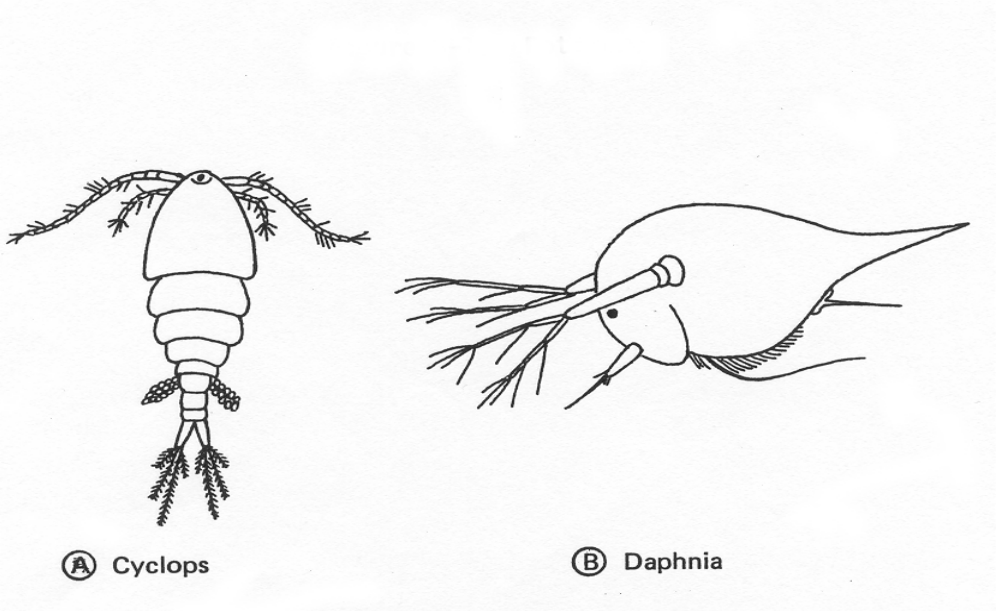


Figure 3-2. Microscopic crustaceans.

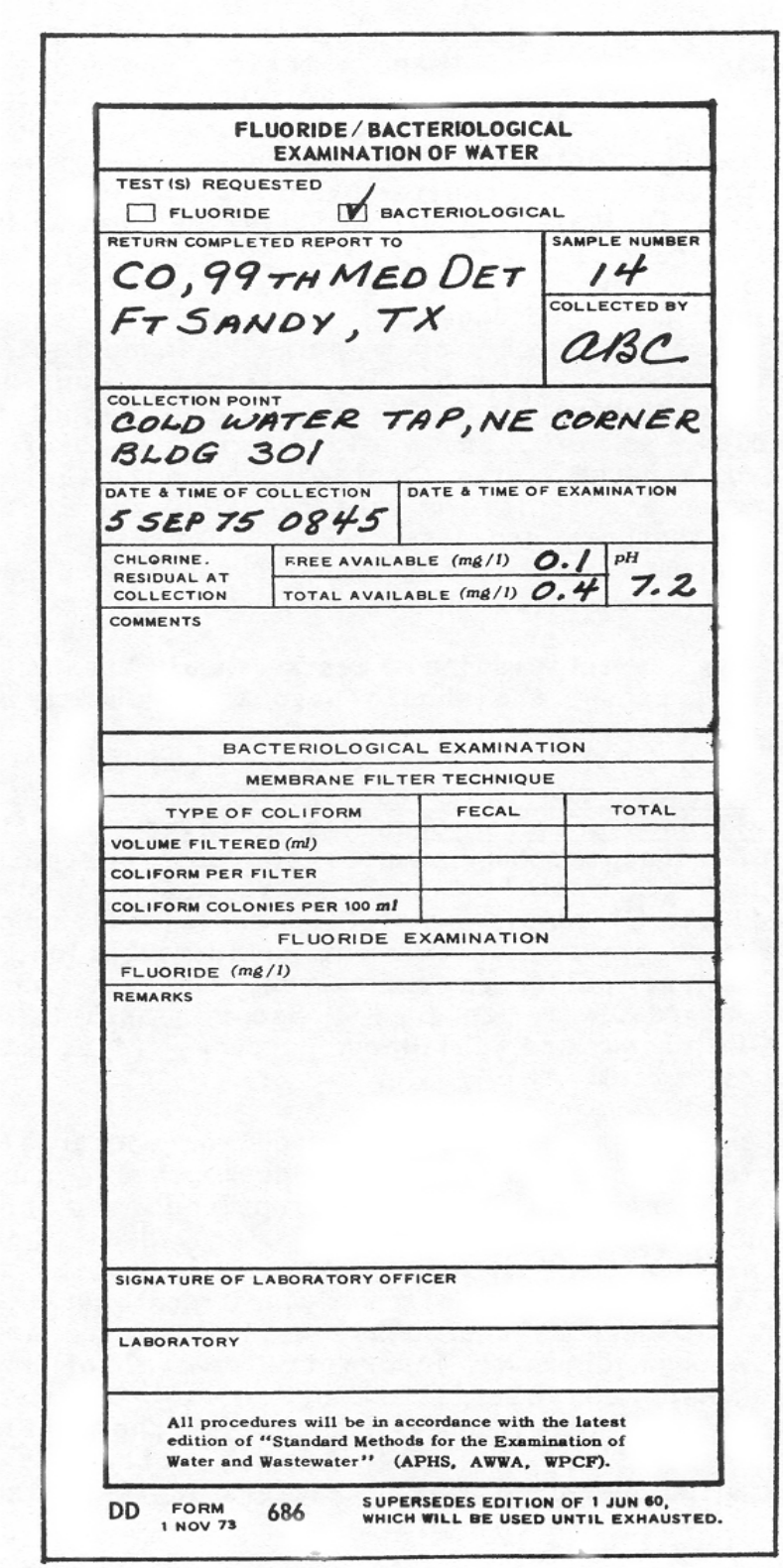
d. **Potability**. Nuclear, biological, and chemical potable water is that which is free of disease-producing organisms, agents, and toxic chemicals. The sanitary microbiologist is concerned with the presence of pathogenic organisms in water. Unfortunately, specific pathogens are not readily detected or identified by normal

bacteriological techniques. Therefore, rather than look for specific' pathogens, the sanitary microbiologist looks for indicator organisms which will indicate the probability that pathogens exist in water. Bacteria of the genera *Escherichia* and *Enterobacter*, known as *coliform* bacteria, are readily identified by their ability to ferment lactose, with the production of gas (subsequently confirmed by other tests). Since *Escherichia* and *Enterobacte*r are normal inhabitants of the human intestinal tract, their presence in water is considered *prima facie* evidence of fecal contamination. Diseases such as cholera, typhoid fever, shigellosis, amebic dysentery, and various helminthic infestations are known to be transmitted through water contaminated with infected human feces. Therefore, the presence of coliform bacteria in water is considered a strong possibility that pathogenic organisms are also present. Accordingly, water that shows the presence of coliforms is not considered potable until it has been treated by disinfection (chlorination).

e. **Sampling**. Bacteriological tests should be made whenever a new source of water is proposed and should be conducted periodically on all existing sources.

(1) Frequency and distribution of samples. TB MED 576, Sanitary Control and Surveillance of Water Supplies at Fixed Installations, prescribes that a minimum of eight monthly samples for coliform determination will be collected at each fixed installation. If the population served is greater than 1,000, an additional sample for 1,000 people will be collected. Sampling points must be representative of the entire installation, and one-fourth of the samples are to be collected each week, so that during the month all points will be progressively covered. Sampling points must be of water distributed for drinking and culinary purposes (that is, dining facilities, hospitals, barracks, and so forth.)

(2) Sample bottles. Bottles used for bacteriological samples may be of any suitable size and shape, but wide-mouthed, ground glass stoppered bottles, of borosylicate glass or polypropylene, are preferred. Metal or plastic screw cap closures may be used provided they are capable of withstanding sterilization without producing toxic or bacteriostatic compounds, or without losing their original configuration and watertight seal. Sample bottles must be thoroughly washed, rinsed in clean (preferably distilled) water enough times to insure the removal of any residual washing compound or detergent, and sterilized by one of the methods described in paragraphs 3-4 and 3-5. The stopper and neck of the bottle are covered with a hood prior to sterilization. Each sample bottle is submitted to the laboratory accompanied by DD Form 686 (Bacteriological Examination of Water) (figure 3-3).



FLUORIDE / BACTERIOLOGICAL EXAMINATION OF WATER

TEST (S) REQUESTED
☐ FLUORIDE ☑ BACTERIOLOGICAL

RETURN COMPLETED REPORT TO
CO, 99TH MED DET
FT SANDY, TX

SAMPLE NUMBER
14

COLLECTED BY
ABC

COLLECTION POINT
COLD WATER TAP, NE CORNER BLDG 301

DATE & TIME OF COLLECTION
5 SEP 75 0845

DATE & TIME OF EXAMINATION

CHLORINE. RESIDUAL AT COLLECTION
FREE AVAILABLE (mg/l) 0.1
TOTAL AVAILABLE (mg/l) 0.4
pH 7.2

COMMENTS

BACTERIOLOGICAL EXAMINATION
MEMBRANE FILTER TECHNIQUE

TYPE OF COLIFORM	FECAL	TOTAL
VOLUME FILTERED (ml)		
COLIFORM PER FILTER		
COLIFORM COLONIES PER 100 ml		

FLUORIDE EXAMINATION
FLUORIDE (mg/l)

REMARKS

SIGNATURE OF LABORATORY OFFICER

LABORATORY

All procedures will be in accordance with the latest edition of "Standard Methods for the Examination of Water and Wastewater" (APHS, AWWA, WPCF).

DD FORM 1 NOV 73 686 SUPERSEDES EDITION OF 1 JUN 60, WHICH WILL BE USED UNTIL EXHAUSTED.

Figure 3-3. DD Form 686 (Bacteriological Examination of Water)

(3) Dechlorination. If the sample to be examined is from a chlorinated water supply, a dechlorinating agent should be added to the sample bottle prior to collecting the sample, in order to stop the bactericidal action of the chlorine and thus give a more accurate indication of the true bacterial content of the sample. Sodium thiosulfate, in an amount sufficient to provide a concentration of 100 mg/1 (0.1 ml of a 10 percent solution of sodium thiosulfate to a 4-oz (118.28 ml) sample bottle), is a satisfactory dechlorinating agent. The sodium thiosulfate must be added prior to sterilization. It is imperative the samples intended for bacteriological examination be delivered or shipped to reach the laboratory with 6 hours after collection. This is important because of the extensive changes that may take place in the bacterial flora even though the samples are stored at temperatures as low as 10ºC. (See paragraph 3-4c)

(4) Procedure. The sample bottle must be kept closed until the moment at which the sample is taken. The stopper and hood are removed in such a manner as to prevent contamination, and the bottle is held near the base. The bottle is filled (leaving an ample air space to facilitate mixing of the sample by shaking) without rinsing and the stopper and hood replaced immediately. The following special procedures apply to specific types of samples.

(a) If the water sample is being taken from a tap, the tap should be connected to a service line from a main, rather than from a storage tank. The tap water should be allowed to run for 2-3 minutes before taking the sample.

(b) If the sample is taken from a well serviced by a pump, the pump should be operated at least long enough for all water to be flushed from the connecting pipes.

(c) Samples taken from rivers, streams, lakes, reservoirs, or other surface sources should be collected at a point which is representative of the water which will be the source of supply to the consumer. Particular caution should be taken not to disturb the bank of a pond or stream; otherwise, fouling of the water may occur and the sample will not be representative. The bottle should be held near the base and plunged, neck downward, below the surface. The bottle is then turned so that the neck points slightly upward and toward the current. If there is no current, one may be created by pushing the bottle, underwater, in the direction of the mouth.

3-2. SEWAGE MICROBIOLOGY

Microbes play an extremely important role in sewage treatment. It is largely through biological digestion that sewage is converted from a highly contaminated, infectious liquid into a relatively stable, inert sludge and a harmless effluent needing only chlorination before it may be discharged into a receiving stream, leaching bed, or other disposal area. There are two biological processes involved in sewage treatment. Aerobic digestion is exemplified by the activated sludge process, in which the wastes from primary settling tanks are thoroughly aerated until active masses of microorganisms settle out as sludge, leaving a clear effluent of relatively low organic

content. A portion of the sludge is returned and mixed with the incoming raw sewage, while the remainder is pumped to digester tanks. Anaerobic digestion is a slower process, which is typified by large digestion tanks, septic tanks, and cesspools.

a. **Aerobic Digestion**. In aerobic digestion, as found in the activated sludge process, the key to successful operation is twofold: providing a continuous supply of oxygen; and preventing excessive variations in raw sewage input, which may upset the balance in the biological population. Aerobic digestion of sewage is a cyclic process. Activated sludge contains bacteria, fungi, protozoa, rotifers, and sometimes nematodes. As raw sewage, high in organic matter, enters the system, bacteria begin to grow in logarithmic proportions. As the bacteria grow, the protozoa, their predators, also grow. When the point is reached at which food is the limiting factor, both bacteria and protozoa begin to die off, forming a floc. Rotifers are able to eat small particles of floc. This floc is subsequently removed in a sedimentation tank. If the process were allowed to continue unaltered, all biological forms would eventually die off. In practice, however, fresh sewage is added only daily and the cycle repeats.

b. **Anaerobic Digestion**. Anaerobic digestion is simpler than the activated sludge process, but it is more sensitive to an imbalance in the biological population. As the sludge in a digestion tank or septic tank settles to the bottom, where there is no free oxygen, the aerobic organisms (aerobic bacteria, fungi, and protozoa) die off or form spores or cysts. Then two distinct biological processes, or phases, occur. Acid-forming bacteria, predominantly facultative anaerobes, begin to metabolize the organic matter, converting it into organic acids, aldehydes, and alcohols. During this first phase (acid phase) the pH is lowered, retarding further bacterial action. The second phase (methane phase) begins when a group of obligate anaerobes begins to increase. These bacteria have the ability to metabolize the organic acids, producing carbon dioxide and methane. The metabolism of amino acids results in the liberation of ammonia, which in turn raises the pH to a level favorable for bacterial growth. As long as a balance in the bacterial flora exists, maintaining the pH at about 6.8-7.4, the digestion process continues with the production of both acids and methanes. Sudden adding of large amounts of solids to the tank may cause an excess of acid production and thereby upset the process by inhibiting the methane-forming bacteria.

Section II. DISINFECTION AND STERILIZATION

3-3. GENERAL

Disinfection and sterilization are indispensable procedures in modern medicine and public health. Without the benefit of these procedures, it would be virtually impossible to carry out many of the routine surgical procedures that are often taken for granted. It would also be impossible to isolate specific microorganisms for their study.

a. **Definitions**.

(1) Disinfection is the destruction of pathogenic organisms. It does not necessarily include the killing of all microbes. A disinfectant is any agent capable of destroying pathogenic microorganisms.

(2) Sterilization is the elimination of all living microorganisms, both pathogenic and harmless. Any object or material free of all microorganisms is referred to as sterile.

(3) Antiseptics are agents which prevent the multiplication of microorganisms but which do not necessarily kill them.

(4) Germicides (bactericides) are agents causing the destruction of microorganisms, both pathogenic and harmless.

(5) Asepsis is the absence of pathogenic microorganisms from a given object or area.

(6) Contamination is the presence of living microorganisms rendering an object or material unfit for its intended use.

(7) Bacteriostasis (virustasis) is the condition by which microorganisms are prevented from multiplying, but in no other way affected.

(8) Sanitization is the reduction in the number of bacteria to a level prescribed as safe by public health or environmental health authorities.

b. **Types of Agents**. Agents for sterilizing and disinfecting may be classified as physical and chemical agents. Specific agents are discussed in paragraphs 3-4 and 3-5.

3-4. PHYSICAL AGENTS

a. **Mechanical Means**. Mechanical means may be used to achieve a high degree of cleanliness and microbe removal, but not to accomplish sterilization. Mechanical means include the following:

(1) Scrubbing. Scrubbing thoroughly with soap and water, and often with an additional chemical, removes microorganisms mechanically, while the soap acts upon them chemically.

(2) Filtration. Filters made of unglazed porcelain, diatomaceous earth, asbestos, and other porous substances are used to remove microorganisms from fluids. Sterility mayor may not be achieved, depending upon the microorganisms present. The smaller viruses and *rickettsiae* are capable of passing through such filters.

(3) Sedimentation. Sedimentation is the process by which bacteria and other particles suspended in a liquid coagulate and settle to the bottom. This process finds its practical application in water purification and sewage treatment.

b. **Heat**. Heat is the most widely used and most effective means of sterilization. All species of microorganisms are killed by heat. The conditions under which the heat is applied, the temperature achieved, the length of time the temperature is maintained, and the species of microorganism involved are all factors affecting sterilization by heat. The temperature that will kill a species of microorganism in 10 minutes is known as the thermal death point. The time required to kill all microorganisms at a given temperature is known as the thermal death time.

(1) Dry heat. Dry heat is used primarily for sterilizing objects made of glass, metal, and substances that are damaged by moisture. For complete sterilization, a temperature of 160º-170ºC (320º-338ºF) must be maintained for at least 1 to 3 hours. Most fabrics are damaged by this much dry heat.

(2) Moist heat. Moist heat provides complete sterilization at lower temperatures and in a shorter time than does dry heat. Whereas dry heat kills by oxidation, a relatively slow process, moist heat kills by coagulation of the protein in the microorganism--a relatively fast process. Moist heat may be applied in three ways.

(a) Boiling. Boiling is the most common means of sterilization. Boiling will kill vegetative forms of pathogenic microorganisms in 5 minutes or less. Spores are much more heat resistant, but they can be destroyed by boiling for 15 minutes. Boiling is not considered a completely effective sterilizing agent, since the spores of certain thermophiles (heat-loving organisms) can survive prolonged boiling.

(b) Steam. Free flowing steam provides about the same degree of effectiveness as boiling, if applied in sufficient volume to maintain a temperature of 100ºC (212ºF).

(c) Steam under pressure. The most effective means of sterilization is by means of the autoclave. The autoclave is an airtight cylinder in which steam may be introduced under pressure. Where as free-flowing steam has a temperature of 100ºC, steam under 15 pounds of pressure per square inch has a temperature of 121.5ºC (251ºF). Steam under 15-20 pounds of pressure per square inch will kill all organisms and spores in 15-45 minutes.

(d) Flaming. Some instruments or objects, such as platinum wire loops used in inoculating laboratory cultures, may be sterilized by holding the object in a gas or alcohol flame until it glows.

(e) Incineration. Incineration is the approved method for sterilizing and disposing of contaminated materials that have no further value. Care must be exercised

that complete combustion of all fragments occurs before any of the residue (ash) is removed from the incinerator.

c. **Cold**. At very low temperatures most microorganisms lose their viability and multiply very slowly, if at all; but most are not killed. Refrigeration has become an indispensable adjunct to the food industry, the medical laboratory, and many other areas in which growth of microorganisms must be inhibited. Some organisms may be killed at low temperatures--a point of importance in the storage, handling, and shipment of laboratory specimens. On the other hand, many microorganisms can be frozen for long periods (as spores) and regain their viability when the temperature is again favorable.

d. **Desiccation**. Natural or artificial drying results in the destruction of most microorganisms. However, bacterial spores resist drying for long periods. In dehydrated foods, bacterial growth does not take place; however, microbial action begins after the foods are reconstituted with water.

e. **Radiation**. Sunlight and artificially produced ultraviolet radiation have a germicidal effect on microorganisms. However, neither one is rapid in its effect nor complete in its action. Both sunlight and ultraviolet lamps are beneficial when used along with other sound procedures for disinfections. X-rays and other ionizing radiation are known to be lethal to microorganisms, but their use for this purpose has not been completely developed.

3-5. CHEMICAL AGENTS

a. **General**. Numerous chemical agents are available as germicides, disinfectants, and antiseptics. Only a few of the principal ones will be discussed here. Antiseptics and disinfectants may act on microorganisms in anyone of or a combination of several ways:

(1) Oxidation.

(2) Hydrolysis.

(3) Combination with cell proteins to form salts.

(4) Coagulation of proteins

(5) Disruption of the cytoplasmic membrane.

(6) Inhibition or inactivation of enzymes.

b. **Phenol**. Phenol (carbolic acid) and its derivatives are among the most useful of the organic germicides. The germicidal efficiency of phenol is used as a standard of comparison for other antiseptics. A five percent aqueous solution of phenol kills

vegetative bacteria readily and the spores more slowly. It is used primarily in disinfecting nonliving objects, because of its harmful effect on tissue. It is particularly useful in disinfecting sputum, blood, feces, and other organic discharges.

c. **Alcohol**. Alcohol is one of the most effective and extensively used antiseptics. It is interesting to note, however, that it is most effective at strength of 70 percent. At concentrations above 90 percent or below 50 percent, it is distinctively less effective.

d. **Halogens**. Iodine and chlorine are two of the best known and widely known disinfectants. Tincture of iodine (two percent in alcohol solution) is used to disinfect cuts and wounds. Strong or old solutions will burn the skin. Chlorine, in the form of sodium or calcium hypochlorite, is used in water purification, in sewage treatment, and in disinfecting food and dairy equipment.

e. **Formaldehyde**. Formaldehyde is a gas used in fumigation. A one percent concentration will kill all pathogenic bacteria in a room or chamber. A 37-40 percent solution in water, known as formalin, is used as a disinfectant, deodorant, and tissue preservative.

f. **Detergents**. Soap and numerous other detergents act upon microorganisms by reducing the surface tension of the water in which they are used, thereby reducing the ability of the microbial cell membrane to control the passage of fluids in and out of the cell by osmosis. Soap tends to neutralize the effect of disinfectants; therefore, it should be thoroughly rinsed off before an antiseptic or disinfectant is applied. *Hexachlorophene*, a phenol derivative, is not affected by soap; therefore, it is incorporated into soaps and detergents used in scrubbing hands of surgical personnel and the skin of surgical patients. Detergents are not true disinfectants, but are used as adjuncts to other agents in order to remove organic matter that might otherwise interfere with the action of the antiseptic or disinfectant. Detergents are also the principal agents employed in sanitizing food service equipment, eating utensils, and patient care items not requiring sterility (bed pans, water pitchers, and so forth).

g. **Antibiotics**. Numerous chemotherapeutic agents, including penicillin, streptomycin, tetracycline, the sulfonamides, and many others, are used to inhibit or kill microorganisms which have already gained entrance into the body of a human or animal host. This usage, however, falls within the realm of therapy, rather than disinfection and sterilization.

Section III. PACKING MICROBIOLOGICAL SPECIMENS FOR SHIPMENT

3-6. GENERAL

The improper collecting and processing of microbiological specimens are often responsible for the failure to isolate and identify the agent responsible for a disease. The specific techniques for collecting various types of specimens do not fall within the

purview of this sub course, but constitute laboratory procedures. However, the environmental health technician should be familiar with the following general considerations:

a. Instruments, containers, and other equipment coming in direct contact with most specimens must be sterile. The stool specimen is one exception.

b. Material for culture must not be exposed to germicides, disinfectant, or other chemicals.

c. Specimens should be properly labeled and dated.

d. Specimens should be delivered to the clinical laboratory immediately after collection.

e. Specimens should be inoculated to media immediately after delivery to the clinical laboratory.

f. To avoid contamination, the specimen should be cultured before making smears or performing special tests.

g. Material for culture should be obtained before the patient receives antibiotic or sulfonamide therapy. If this is not possible, the type of therapy should be indicated on the microbiology request form. SF 553 (Microbiology I) (figure 3-4) is used for most microbiology requests. SF 554 (Microbiology II) is used for suspected viral, fungal, and acid-fast bacterial infections.

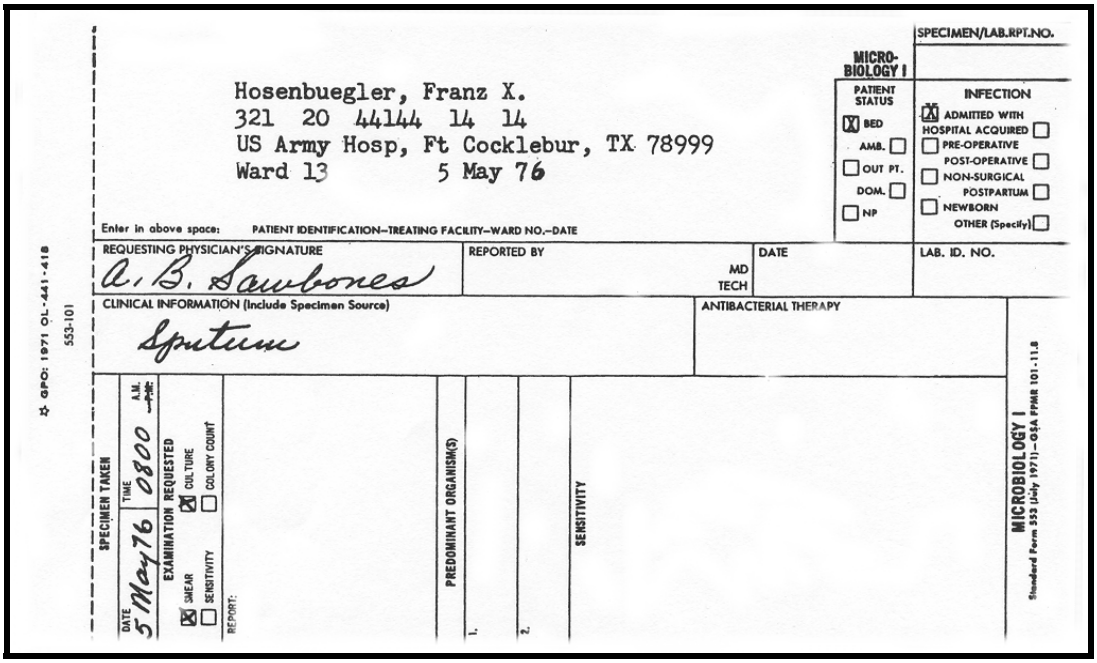


Figure 3-4. SF 553 (Microbiology I)

h. If anaerobic cultures are requested, the specimen should be collected and transported in the manner prescribed by the laboratory.

3-7. IMPORTATION AND INTERSTATE SHIPMENT OF HUMAN PATHOGENS AND RELATED MATERIALS

It is frequently necessary to ship specimens to reference laboratories for analysis or confirmation. Acceptable shipping containers and procedures for packing specimens are described below:

a. **Noninfectious Specimen**.

(1) Enclose the specimen in a sterile glass container and cap. A screw-capped test tube or jar is satisfactory.

(2) Place the glass container in standard double shipping containers.

(3) Pad the spaces between the containers to guard against breakage.

(4) The properly packed container of noninfectious material will have the following layers, from specimen outward: specimen, glass, padding, metal, padding, and heavy cardboard (figure 3-5(A)).

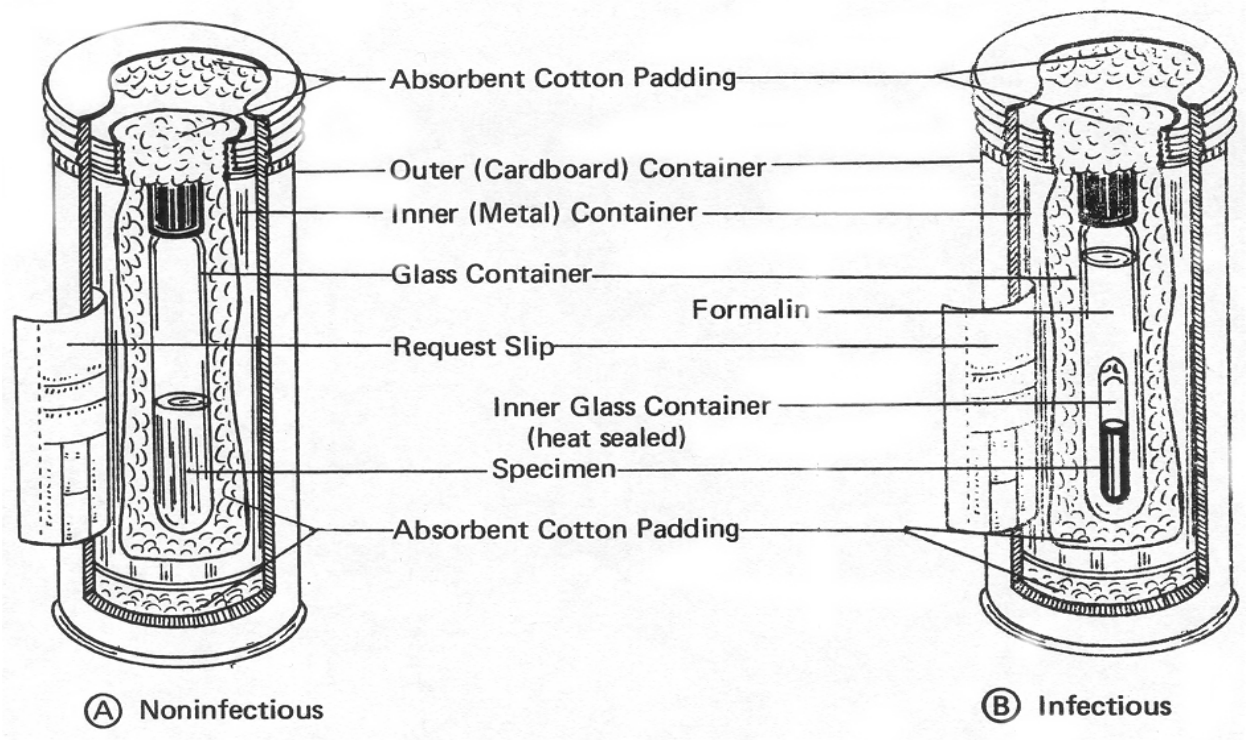


Figure 3-5. Microbiological specimens picked for shipment.

b. **Infectious Specimen**.

(1) Enclose the specimen in a sterile, stout glass tube, and seal the ends of the tube by fusion of the glass.

(2) Place the glass tube containing the specimen in a stout glass container that can be sealed by an insulated screw cap, rubber stopper, or by fusion of the glass.

(3) Add formalin to the outer container so that the inner glass container is surrounded by formalin; then seal the glass container with wax. This procedure provides for disinfection's if the inner container should break.

(4) Pack this double glass container in standard double shipping containers.

(5) Pad the spaces between the containers to guard against breakage.

(6) The properly packed container of infectious material will have the following layers, from specimen outward: specimen, glass, formalin, glass, padding, metal, padding, and heavy cardboard (figure 3-5 (B)).

c. **Additional Requirements**.

(1) The importation or subsequent receipt of etiologic agents and vectors of human disease is subject to the Public Health Service Foreign Quarantine Regulations (42 CFR, Section 71.156). Permits authorizing the importation or receipt of regulated materials and specifying conditions under which the agent or vector is shipped, handled, and used are issued by the Centers for Disease Control.

(2) The interstate shipment of indigenous etiologic agents, diagnostic specimens, and biological products is subject to applicable packaging, labeling, and shipping requirements of the Interstate Shipment of Etiologic Agents (42 CFR Part 72). Packaging and labeling requirements for interstate shipment of etiologic agents are summarized and illustrated in figure 3-5.

(3) Additional information on the importation and interstate shipment of etiologic agents of human disease and other related materials that may be obtained by writing to:

Centers for Disease Control
Attention: Office of Biosafety
1600 Clifton Road, N.E.
Atlanta, Georgia 30333
Telephone: (404) 329-3883
FTS: 236-3883

Continue with Exercises

EXERCISES, LESSON 3

INSTRUCTIONS. The following exercises are to be answered by marking the lettered response that best answers the question or by completing the incomplete statement or by writing the answer in the space provided at the end of the question.

After you have completed all the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers.

1. Crustaceans are important in the sanitation of natural bodies of water in that they help to control _____.

2. The presence of rotifers in water is an indication of _____ pollution levels.

   a. Low.

   b. High.

3. Bacteria of the genera *Escherichia* and *Enterobacter* are known as _____ _____ bacteria.

4. The activated sludge process in sewage treatment is based upon _____ _____digestion.

5. In anaerobic sewage digestion, _____ forming bacteria predominate in the first stage, and _____forming bacteria become active in the second stage.

6. The septic tank is an example of _____ digestion.

7. The destruction of all microorganisms is known as _____.

8. The destruction of all pathogenic organisms id known as _____.

9. Microorganisms may be destroyed by either _____ or _____ agents.

10. The most widely used and effective means of sterilization is _____.

11. The thermal death point is the temperature at which a specific organism will be kill after _____ minutes of exposure.

12. Dry heat requires _____ temperatures than moist heat to accomplish sterilization.

    a. Higher.

    b. Lower.

13. Moist heat requires a _____ exposure time than dry heat for complete sterilization.

    a. Longer.

    b. Shorter.

14. The most common means of sterilization are _____.

15. Which of the following statements is valid?

    a. Sub-freezing temperatures are effective as a means of sterilization.

    b. Very low temperatures inhibit the growth and multiplication of microorganisms, but usually do not kill the microbes.

16. The standard for measuring the germicidal effectiveness of antiseptics is that of _____.

17. Alcohol is most effective as a disinfectant when applied in a concentration of _____ percent.

18. The two halogens normally used as disinfectants are _____ and _____.

19. Detergents accomplish sanitization primarily through the process of reducing _____.

20. A 37-40 percent solution of formaldehyde gas in water is known as _____.

21. Instruments, containers, and other equipment coming in contact with microbiological specimens must be _____.

22. List, in order, the layers from the center outward of a microbiological specimen properly packed for shipment:

a. Infectious	b. Noninfectious
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 3

1. Algae. (para 3-1c(2))
2. a (para 3-1c(1))
3. Coliform. (para 3-1d)
4. Aerobic. (para 3-2a)
5. Acid-, methane. (para 3-2b)
6. Anaerobic. (para 3-2b)
7. Sterilization. (para 3-3a(2))
8. Disinfection. (para 3-3a(1))
9. Physical, chemical. (para's 3-3, 3-4, 3-5)
10. Heat. (para 3-4b)
11. 10. (para 3-4b)
12. a (para 3-4b(2))
13. b (para 3-4b(2)
14. Boiling. (para 3-4b(2a))
15. b (para 3-4c)
16. Phenol. (para 3-5b)
17. 70. (para 3-5c)
18. Iodine, chlorine. (para 3-5d)
19. Surface tension. (para 3-5f)
20. Formalin. (para 3-5e)
21. Sterile. (para 3-6a)

22. a. Specimen.
Glass.
Formalin.
Glass.
Padding.
Metal.
Padding.
Cardboard.

b. Specimen. (para 3-7, figure 3-5)
Glass
Padding
Metal
Padding
Cardboard

End of Lesson 3